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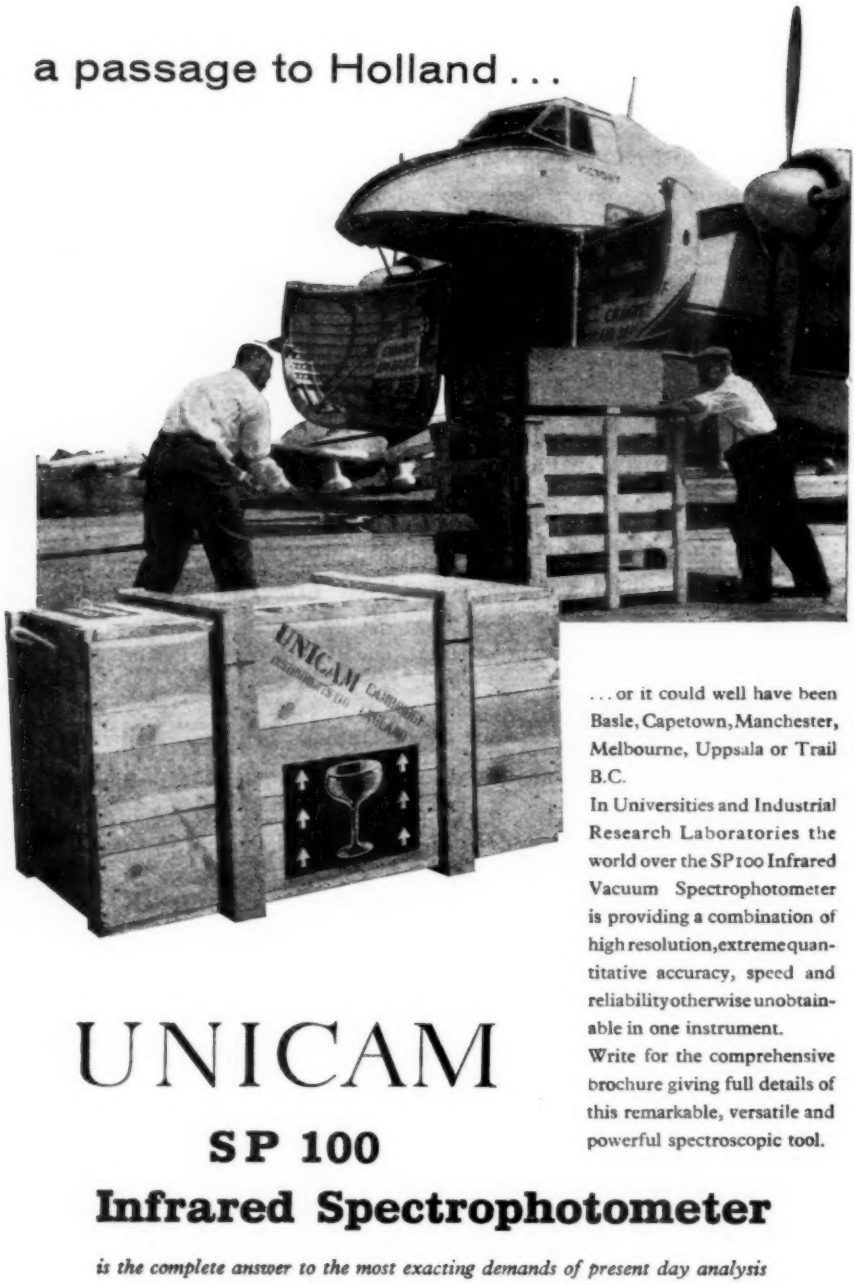

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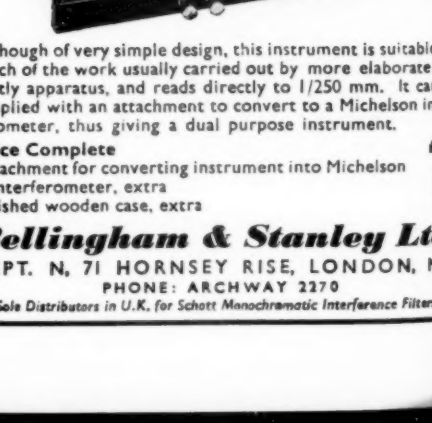

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INDUSTRIAL RELATIONS IN THE WELFARE STATE

IN his book, *Nationalized Industry and Public Ownership*, published last year, in dealing with labour relations, Prof. W. A. Robson wrote that those who emphasized the need to imbue the employees of public corporations with the spirit of public service were right from every point of view. Without it, nationalization cannot succeed, but we must not expect that nationalization by itself will transform the outlook or influence the motives of those who work in an industry. What we in Britain should seek is to awaken in the work-people a strong desire to serve the public as well as they possibly can, together with the desire to improve their own standard of living.

Prof. Robson recognized then quite frankly that for such aims to be realized the unions must be willing to abandon some of their most cherished prejudices and to change some of their obsolete practices; that they need to abandon their defensive negative, demanding attitude, conditioned largely by the capitalism which produced them, and display qualities of constructive leadership, understanding and co-operation with management. In this way they can respond to the challenge to co-operate in the administration of vast industries operated for the benefit of the community. Especially, he added, the unions need to improve the quality of their own officers. Even the largest and wealthiest unions are handicapped by a lack of skilled staff—economists, statisticians and research workers. They seem reluctant to appoint men who have had a university education, even when they are their own members who have won scholarships paid for by the union.

In the past year it has become more and more apparent that the unions, as Prof. Robson wrote, must consider the part they have to play in the sphere of nationalized industries, and recognize the need to revise their own organization, attitudes and practices so as to enable them to play it more effectively. Nor is this need confined to the nationalized industries. It is manifestly true of the shipping and shipbuilding industries, as was fully recognized in a debate on those industries in the House of Commons in July.

An admirable exposition of the function of labour and the trade unions in industry and commerce is contained in a paper by Mr. W. J. Carron, president of the Amalgamated Engineering Union, read before the Royal Society of Arts last December and now published (*J. Roy. Soc. Arts*, 109, 248; 1961). Mr. Carron recognized the difficulty of apathy among union members, but here, as in the matter of restrictive practices, co-operation is necessary; and the better the understanding between the two sides of industry the more readily restrictive practices on either side, and perhaps also apathy, are likely to be removed. If there is one thing in fact that recent economic debates have emphasized, it is that trade unions, no less than other groups, whether scientists, technologists, teachers or others, are all part of the community and suffer with it when one section, luckier or less scrupulous than the rest, takes more than its fair share.

What is needed in fact is, as was admitted in the House of Commons, a climate of opinion which recognizes that higher wages or salaries must be earned and related to productivity; a system enlisting the co-operation of both trade unions and employers, making it in the interest of the unions to eliminate restrictive practices and the employers to show the maximum enterprise. To create such a climate of opinion or understanding is a major task, and might well be described as one objective of the industrial code which Lord Fleck advocated in his presidential address to the Industrial Co-partnership Association on July 9 and in a speech in the House of Lords on June 14. Studies such as that of Mr. D. F. Macdonald on *The State and the Trade Unions** are to be welcomed for the same reason, since they contribute to an impartial appreciation both of past mistakes and the action which now needs to be taken. Mr. Macdonald brings out particularly well the changed position of the unions in the social scene to-day, and the way in which the intimate concern to the community of their work and methods has stimulated such close scrutiny.

Mr. Macdonald does not intend to offer any constructive proposals. He shows that the trade unions cannot afford to ignore any attempts at a wage-policy and to condemn or belittle an independent body which studies and makes pronouncements on the wages question as part of a review of national economic considerations. The carping at the Council on Prices, Productivity and Income has probably done more harm to its trade union critics in the eyes of the public than to the prestige of the Council. Mr. Macdonald recognizes also that the principle of compulsory membership, or 'the closed shop', may call for safeguards to protect the individual as well as the public interest against group pressure; but he does not discuss the safeguards which from time to time have been suggested, and which might both safeguard the individual and offer a more effective redress of grievances that under present conditions are liable to result in unofficial strikes.

Some of these problems were considered in a debate on industrial relations in the House of Commons on March 17, when Mr. R. Marsh, in opening the debate, suggested that a rationalized trade union structure must precede the creation of a wages structure. He

* The State and the Trade Unions. By D. F. Macdonald. Pp. vii + 199. (London: Macmillan and Co., Ltd.; New York: St. Martin's Press, Inc., 1960.) 21*s*. net.

also maintained that most of the problems arose from a lack of joint consultation and of understanding on both sides. Sir Richard Glyn agreed that a more rational trade union movement is essential for a planned industry and a planned economy, but probably the keynote of this debate was the stress laid by both sides of the House on training for management. The right type of management—the right kind of men and the right kind of spirit—is an essential element. Although the limitations of management were equally recognized in this constructive debate, it was clear that given management of this type, there would be no failure to tackle the problem of re-training skilled men whose skills were made obsolete or redundant through technological advance.

The recognition of the importance of sound industrial relations to the economic life of Britain and to the maintenance and improvement of the British standard of living obviously lies behind the idea of an industrial code which Lord Fleck proposed in the House of Lords debate on co-operation in industry. Earlier, in his Fawley Lecture, Lord Fleck had suggested that if at least ten universities, including colleges of advanced technology, were to develop departments specifically concerned to promote good industrial relations, it would be worth while attempting from such sources to draft a broad measure of industrial philosophy. Once again, in the debate in the House of Lords, there was a good deal of stress on education on both sides, Viscount Amory calling for positive and dynamic co-operation and team-work, for spontaneous respect and concern for efficiency, and for pride in the job, both individually and collectively; and Lord Citrine, who doubted whether there are really any new methods open to us in combating apathy, suspicion and hostility in this field, thought that the decisive factor is to re-establish the leadership of the Trades Union Congress.

This large measure of agreement on both sides as to the need for a fuller understanding of these problems, for a climate of opinion in which more effective co-operation could flourish and for inspired leadership was reflected in subsequent debates on the economic situation in both Houses of Parliament. Mr. Hugh Gaitskell, in a notable speech on July 18, appealed for an attack on the complacency of some managements and the appalling indifference of some workers, and the conditions which foster such situations. He recognized that this was much harder than the adoption of a greater measure of planning, the more positive attitude to particular industries, or the increased degree of professionalism in business and exporting on which he also insisted. To some extent the Government has already moved in this direction, but it cannot fairly be claimed that the Government has yet provided the leadership which is needed and which was even more powerfully adumbrated by Mr. Harold Wilson in speaking on the economic situation on July 26. The necessary climate of opinion is not to be created by appeals to materialism, selfish acquisitiveness, or social emulation. There must, he argued, be some planning, but we need also a sustained increase in production and greater determination to control rising costs; and he admitted frankly that we cannot afford, year by year, an increase in economic rewards—profits, salaries, rents or wages—greater than the increase in productivity which provides the means to all rewards.

This is, of course, an argument for giving much higher priority to increased productivity, and Mr. Wilson believes that it is well within the capacity of Britain to achieve an increase, year by year, of 4 or 5 per cent in productivity and of 4 or 5 per cent in wages. It was as part of a wide attack on the spirit of lethargy which pervades too much of British industry that he advocated the ending of restrictive practices wherever they occur which hold back the national inventiveness and skill, and it is in this context that the trade unions must be regarded to-day. Mr. Nigel Birch, in the same debate, made an equally forthright appeal for an attack on industrial inefficiency, wherever encountered, and for a policy of encouraging understanding by the fullest possible publicity. Mr. R. Carr also stressed this need for the consistent and clear exposition of policy and for a far-reaching attack on restrictive practices. He made, too, a number of constructive suggestions for dealing with the shortage of skilled labour and encouraging companies which are progressive and adventurous, and some of these suggestions were welcomed on the Opposition side of the House.

These suggestions, however, are scarcely relevant in the present context. What is relevant, however, is the measure of agreement between political parties as to objectives and as to the need for the Trades Union Congress to play its part and secure the shedding by the unions of outmoded practices and ideas. That is admittedly essentially a matter for the unions themselves, and whether Parliament can do anything to help, that can only be done with their co-operation. In his classic work on *The English Constitution*, Walter Bagehot wrote of the House of Commons that its teaching function was the one which was worse performed: "A great and open council of considerable men cannot be placed in the middle of a society without altering that society. It ought to alter it for the better. It ought to teach the nation what it does not know".

In some of its recent debates—on science and on economic affairs, for example—the House of Commons has shown the extent to which, even in the conditions of to-day, it can exercise that educative function. It can still do much to create public understanding. It could go far to create the climate of opinion which will accept the harsh measures that may be needed. When leadership is not forthcoming from the Government, it may well be able once again to stimulate it as well as elicit the response required to sustain the measures of policy that a situation demands. Further, the trade unions themselves may well provide a classic example of the way in which Parliament through its educative function may stimulate both the leadership needed and the support which will make the appropriate measures effective. At a time

like this, the Parliamentary and Scientific Committee has something to contribute to this educative function of Parliament. It is partly a means of informing Parliament, and professional men should be careful to use it responsibly and imaginatively to the maximum extent; but it is also a means through which Parliament can exercise that function in a wider if specialized field.

BALLISTIC MISSILE AND SPACE TECHNOLOGY

Ballistic Missile and Space Technology

Proceedings of the Fifth Symposium held at Los Angeles, California, August 1960. Edited by Donald P. LeGalley. Vol. 1: Bioastronautics and Electronics, and Invited Addresses. Pp. xiii+494. Vol. 2: Propulsion and Auxiliary Power Systems. Pp. x+439. Vol. 3: Guidance, Navigation, Tracking, and Space Physics. Pp. x+450. Vol. 4: Re-entry and Vehicle Design. Pp. x+422. (New York: Academic Press, Inc.; London: Academic Press, Inc. (London), Ltd., 1960.) 9 dollars per volume; 36 dollars per set.

A SERIES of symposia on ballistic missile technology was started in June 1946 by the Ramo-Wooldridge Corporation and the Ballistic Missile Division of the United States Air Force; the fifth symposium was held in August 1960 at the University of Southern California in Los Angeles. To those who know the extensive campus of this University and the enormous size of the United States missile industry it will be no surprise to learn that more than one thousand scientists and engineers from all parts of the United States attended this symposium, at which seventy-four unclassified and fifty-one classified papers were presented after selection from two hundred and fifty-five manuscripts submitted. It has become a feature of the United States' so-called 'aerospace' programme that huge gatherings of scientists come together at frequent intervals for the presentation of papers covering a field so vast that it is obvious that no single participant could appreciate more than a small fraction of the specialized subjects involved. Since most of the time at symposia is spent in discussion outside the formal sessions, this plethora of papers is perhaps of little importance. Another feature is, however, of more serious significance, and the four volumes which report in full the proceedings last year exhibit it only too clearly. The frequency and size of these gatherings are now so great that there are just not enough papers of good quality to go round; difficulties with military security no doubt also play a part. The result is a number of contributions of limited interest, which explore in detail some byway which only the author really wishes to tread.

Nevertheless, the present volumes of proceedings are, in total content, of considerable interest and value to workers in missile and satellite technology. This is especially so as the editor and publisher succeeded in producing them within a very few months of the actual conference, thus allowing us to gain an up-to-date view of the extremely varied topics which are the concern of the industrial, government and university research laboratories and development teams engaged in U.S. ballistic missile and space activities. The emphasis throughout is towards space topics; this is shown by the fact that only twenty-two out of the seventy-four papers deal with subjects which have any significant relevance to non-space missile applications. Few of the authors take a basic approach to their subject although here and there one finds interesting items such as the study reported in Vol. 1 of an experimental investigation of gyro spin-axis bearing lubrication; or the careful theoretical treatment of the equations of motion of a satellite in a gravitational field with particular reference to attitude stabilization and control system analysis in Vol. 3. Vol. 1 is devoted to bioastronautics and electronics and includes also the general addresses given to the symposium by such prominent speakers as Dr. T. Keith Glennan, John H. Rubel, General Bernard Schriever and Dr. Simon Ramo. A topical note is struck by Ramo, in a style familiar to all who have known him during the past fifteen years in the missile business, by his paper on "The Life and Times of the Crash Program". This diverts attention from missiles to the great and growing need for improvements in United States' educational methods, the shortage of teachers and the potential applications of electronic teaching devices for technical subjects; one awaits the howl of protest from protagonists of the humanities against this further indication of the narrowness of scientific educational methods. Vol. 2 covers propulsion and auxiliary power systems; Vol. 3 guidance, navigation and space physics. Re-entry and vehicle-design problems are dealt with in Vol. 4.

The text is reproduced by the photo-lithographic process; illustrations and diagrams are clear and graphs are particularly well reproduced with scales which allow accurate interpolation. W. H. STEPHENS

TECHNOLOGY OF CHEMICAL PROCESSING

Encyclopedia of Chemical Technology

Edited by Raymond E. Kirk and Donald F. Othmer. Second Supplement Volume edited by Anthony Standen. Pp. xi+970. (New York: Interscience Publishers, Inc.; London: Interscience Publishers, Ltd., 1960.) 175*s*.

CHEMICAL technology preserves its position at the spearhead of advancing standards of living. Not only has man distinguished himself as a toolmaker, but also he is an enthusiastic converter of the materials in his environment. Chemical reaction, chemical plant and processing are all basic to this vast enterprise of our era.

The very range of chemical technology makes it extremely difficult for a worker to find readily information related directly or indirectly to his pursuit. Established work in physics, chemistry, applied mathematics and even biological science is of interest to him. Knowledge of these is likely to result in unexpected innovation. For example, the theory of turbulent diffusion has enabled those concerned with the dynamics of chemical reactors to refine the calculations for their designs. Often such refinements lead to simpler methods of operation, better yields and improved quality of products.

As a compendium of information for chemical technologists, the Kirk–Othmer encyclopædia is maintaining its traditional calibre, with the appear-

ance of a second supplementary volume. The function of these supplements is to deal with recent developments. This is done in a comprehensive yet fairly readable manner. The range of material and level of appeal are extensive. Fundamental theory is presented; so too are various chemical processes, modern unit operations, techniques for analysing problems in processing, and there is also information which should be of interest to the technologist concerned with economics and planning. Since there are fifty-eight articles, a few selected more or less at random will help to illustrate the diversity.

The comparatively new field of irreversible thermodynamics is discussed by H. Balescu, very appropriately, since the Belgian school has been very active in promoting this work. He shows how the equations of change are used together with Gibbs's fundamental formula of classical thermodynamics in deriving a formulation for production of entropy. This expression, bilinear in the forces and the flows caused by them, is the central concept in irreversible thermodynamics. Onsager's phenomenological principle is described, followed by a section on application. We see that in cases where chemical kinetics becomes so complicated that it can no longer be used exactly, thermodynamics can be a valuable tool in providing a relationship between rate-constants.

A chemical process of great technological importance these days is the manufacture of halogenated butyl rubber. This confers properties such as resistance to degrading by oxidation, adherence and ability to co-vulcanize with other polymers. Yields and mechanisms of reaction are described for processes involving various halogenating agents; methods of curing with zinc oxide and amines are discussed.

Another commercial enterprise of note is the manufacture of polypropylene. Production of crystalline polymers on an industrial scale began toward the end of 1957. Chemical and physical properties are discussed; a very useful table shows a comparison of polypropylene and high-density polyethylene. In the section on Ziegler-type catalysts it is interesting to note that the question whether growth of polymer occurs on the transition metal or on aluminium is still obscure. Effects of processing variables on polymerization are discussed; so too are techniques of moulding and extruding the final products.

A unit operation which promises to become a standard technique in chemical technology is that of separation by diffusion. An article deals with separation, usually of helium or hydrogen, by diffusion through membranes composed of a substance in the glassy state. The theoretical treatment is particularly good.

The use of radioisotopes for analysing the operation of chemical processes is described. Apart from the determination of levels of liquids or fluidized solids in vessels, continuous chemical analyses can be carried out; they are also used to detect leaks in buried pipes or in heat exchangers. Another application is to investigate the extent of reverse flow in chemical reactors.

An example of information useful to the industrial economist is an article on synthesis gas. After a description of various processes, which includes typical data indicating performance of the gas, the investment needed to produce hydrogen is shown graphically as a function of rates of production.

This supplementary volume certainly has a wealth of information, and may be recommended to the chemical technologist, whatever his pursuit. It is a pity that 'technologese' is not avoided throughout the book; one flinches, for example, at reading the word 'recognization'.

R. H. Villet

AMINO-ACIDS

Chemistry of the Amino Acids

By Jesse P. Greenstein and Milton Winitz. Vol. 1: Pp. xxi + 1–760*d*. Vol. 2: Pp. xi + 761–1816*b*. Vol. 3: Pp. xiii + 1817–2872. (New York and London: John Wiley and Sons, Inc., 1961.) £40 the three volumes.

A NEW comprehensive treatise of more than 2,800 pages on the chemistry of amino-acids is a major event in the chemical and biochemical literature. The first text-book in the English language specializing on amino-acids was *The Biochemistry of the Amino Acids*, by Mitchell and Hamilton, published in 1929. It was a book of 600 pages, and more than half of it was concerned with physiology and biochemistry. Nine years later its place was taken by *The Chemistry of Amino Acids and Proteins*, edited by C. L. A. Schmidt, a treatise of a little more than a thousand pages written by a team of eighteen authors. In 1951, D. M. Greenberg edited a similar multi-author text-book on amino-acids and proteins of about 900 pages which included the metabolic aspects. After an interval of ten years in which the literature has grown at an exceedingly rapid rate, the late Dr. Jesse P. Greenstein, in collaboration with Dr. Milton Winitz, has written a new monumental work, arranged in three volumes and fifty-two chapters. The approach is essentially that of the organic chemist, and unlike its predecessors it deliberately omits most aspects of metabolism as this field has been recently dealt with in Dr. Alton Meister's book published not long ago from Dr. Greenstein's laboratory.

The bulk of the work deals with the physiologically occurring amino-acids but a number of related substances are also discussed, and apart from the purely chemical features a few physiological aspects, for example renal clearance and active transport, are included. The first of the three volumes deals with the general and physical chemistry of amino-acids. There is a full discussion of nomenclature, classification and configuration. The physical chemistry covers *inter alia* the dipolar ion concept, thermodynamics and solubility. Other chemical features which are extensively described are the metal complexes in which amino-acids take part, salt formation, effects of radiation, the general principles of α-amino-acid synthesis and of the resolution of isomers. The second volume covers chemical procedures for the synthesis of peptides, the analytical procedures including manometry, titration methods, isotope dilution, chromatography, micro-biological assay and spectrophotometry. The sequential analysis of peptides is also extensively treated. One chapter deals with the enzymes involved in the determination, characterization and preparation of amino-acids. The third volume is mainly concerned with a description of the preparation of the individual amino-acids. Apart from the protein-bound amino-acids there is an exhaustive treatment of other amino-acids and their derivatives. These include the large number of new amino-acids which have been discovered in biological

material in recent years, thanks largely due to the chromatographic techniques and to the isolation of numerous special peptides and proteins produced by micro-organisms (for example, antibiotics).

The senior author unfortunately did not live to see the publication of this book. He died suddenly in February 1959 as a result of a cerebral hæmorrhage when the book had been virtually completed. A brief biographical sketch by the junior author prefaces the book and imparts something of the unusual gifts of the mind and character of the senior author. The book bears throughout the mark of Dr. Greenstein's distinguished style, outstanding in its clarity, accuracy, liveliness, elegance and beauty. A sample of this, conveying also the intentions of the authors, is the following passage from the preface:

"Let it be admitted that we have been entranced by the spectacle of these many and diverse phenomena. In attempting to transfer these pleasurable emotions to paper, and to portray accurately the work of so many able investigators, we were well aware of the magnitude and difficulty of the task which we set for ourselves, and of the impossibility of perfection. With filial piety and in justice to those who have built this field, as well as to convey a sense of perspective, we have stressed where we could the historical development of each subject. The literature of science is generally curt and colorless, except when enlivened at times by the statement of controversial opinion; on occasion an expression of notable and graceful generosity or of far-seeing imagination finds its way into this literature, and such examples have been quoted where pertinent in the pages to follow. The topics covered in this work have generally been those wherein a certain stability has been attained and are thereby suitable for comprehensive treatment—not that progress has ceased in such topics, but rather that nothing has so far emerged to render any substantial portion of them obsolete or questionable."

The references to the historical developments and the quotations from earlier writers are a particular asset of the book. Those from foreign languages are given in the original, with a translation in an appendix of the book. They add to the enjoyment and interest of a standard work which for many years to come will be an invaluable source-book. H. A. KREBS

INORGANIC CHEMISTRY

Inorganic Chemistry

By Jacob Kleinberg, William J. Argersinger, Jr., and Ernest Griswold. Pp. viii+680. (Boston: D. C. Heath and Company; London: George G. Harrap and Co., Ltd., 1960.) 75*s*. net.

THE past few years have witnessed radical changes in the teaching of inorganic chemistry to undergraduate classes. These may largely be attributed to a fruitful interaction, at the research level, between physical and theoretical chemistry on one hand, and descriptive inorganic chemistry on the other. It has thus been possible to stimulate the undergraduate by presenting inorganic chemistry as a subject which makes full demands on his powers of comprehension and reasoning.

A new single-volume text on inorganic chemistry is therefore to be welcomed at this time. Drs. Kleinberg, Argersinger and Griswold have attempted to tackle the subject so as to reflect these present-day changes in its teaching. To this end, the book is divided into three parts. Part 1 (211 pages) is devoted to a brief account of the development of atomic theory, to elementary quantum mechanics, then on to the problem of the electronic configuration of atoms and the consequence of this on chemical and physical properties, especially from the point of view of chemical periodicity, and finally a section on chemical binding and molecular structure. Part 2 (80 pages) is entitled "Some Aspects of Chemical Behaviour", and consists of two chapters, on co-ordination compounds and a particularly good one on acid-base relationships and reactions in non-aqueous systems. Part 3 is divided into three sections. The first deals with the representative elements (196 pages), the second (136 pages) with the transition elements (the descriptive chemistry is presented here with more detail than is usual), and the third (58 pages) with the lanthanides and actinides.

The authors have deliberately chosen to omit sections on nuclear chemistry, the inert gases, and organo-metallic compounds. The last of these topics was considered to be one which, in order to have covered it adequately, would have increased the scope and size of the text beyond limits that they had set themselves. I feel that this decision was unfortunate. The field of organo-metallic chemistry is one which has in many ways been in the forefront in the recently much talked of renaissance of inorganic chemistry, and its inclusion in this text need not have occupied more than 30 or 40 pages. I would also have wished to see sections on crystal chemistry, physical methods of structure-determination (for example, infra-red, visible, and ultra-violet spectra are rarely mentioned), and reaction mechanisms. Very few of the discoveries or developments of the past five years are included. The problem of bonding is dealt with almost wholly in terms of valence bonds and the treatment is obsolete in many instances. In addition, it would have been preferable to have introduced much more physical and theoretical chemistry into Part 3 and thus emphasize the essential unity of the subject.

The authors are to be congratulated on the general presentation of their book, which is well written. It can, with few reservations, be recommended for use at least up to Part 1 honours B.Sc. standard.

M. F. LAPPERT

A MUCH-STUDIED PROTOZOON

The Biology of Stentor

By Vance Tartar. (International Series of Monographs on Pure and Applied Biology. Division: Zoology, Vol. 5.) Pp. x+413. (London and New York: Pergamon Press, 1961.) 75*s*. net.

THE ciliate protozoon *Stentor* is large enough to be operated on by hand (with skill) and is remarkably amenable to surgery. For these reasons it has been the object of numerous morphogenetic studies, culminating in Tartar's own outstanding contribution. As the author of this book, Tartar shows his over-riding interest in cell morphogenesis.

Fission, re-organization (the "physiological regeneration" of Schwartz) and regeneration are related, in that each involves the formation of new adoral membranellæ (which appear as a primordium) and mouth structures and the fusion and renodulation

of the macronucleus. Although the study of *Stentor* has contributed important information about the mechanism of cleavage, the events leading to fission have not been elucidated. More is known about re-organization and regeneration. Tartar rejects several theories and concludes with Schwartz that re-organization is induced by a certain departure from the normal macronucleo-cytoplasmic ratio and permits a re-adjustment. He does not reveal whether this re-adjustment is merely one of area, or how it affects the quantities of deoxyribonucleic acid and other macronuclear constituents. Regeneration is induced by damage to the feeding organelles or when these are defective. Tartar interprets grafting experiments in terms of two cytoplasmic states: activation, which favours the formation of a primordium, and inhibition, which blocks this process. The fusion and renodulation of the macronucleus and mitosis of the micronuclei are considered by Tartar to be set in motion by the onset of cytoplasmic activation, for which the presence of the macronucleus is itself necessary. There is some evidence to suggest that the differentiation of mouth parts at the posterior end of the primordium is induced by the surrounding protoplasm, probably in relation to a gradient.

Perhaps Tartar's greatest achievement concerns the induction of the primordium itself. The precise localization of the primordium in relation to the pattern of stripes in the ectoplasm had already been noted by earlier workers, but Tartar has postulated that a primordium is formed in a region of contrast in stripe width, and has adduced effective experimental evidence in support of this hypothesis. This brings us back to gradients within the cell cortex, and so to the kinetosomes, linearly ordered, prolonged outwards as cilia, presumably themselves the site of important aspects of ciliary activity, and thus perhaps the dominant feature in cell gradients and 'molecular ecology'. All these and other aspects of morphogenesis are treated very fully; Tartar's book is of the nature of a detailed, lucid, and comprehensive review of these topics.

What then of other aspects of the biology of *Stentor*? These are in general summarized very competently and usefully from the literature, though with something slightly less than the critical approach of the cytophysiologist. Even the arrangement is peculiar. Behaviour is treated rather well near the beginning, followed by fine structure, including contractility; but ciliary co-ordination is exiled to a hotch-potch chapter far removed. Some important aspects of fine structure are summarized in a single stereogram; but it is doubtful whether the reader can appreciate or assess the evidence without electron micrographs such as those by Randall and Jackson. Sleigh's work on membranellar beat is faithfully retailed, but the co-ordination of body cilia is quite inadequately discussed, with no mention of the possibility of surface depolarization and no reference to pertinent work on other Protozoa.

Considerable space is given to the function of the macronucleus and to interspecific grafts, and useful comparisons are drawn with experiments on amoebae. The species of *Stentor* are briefly but usefully described and are illustrated in colour. In the penultimate chapter the simple secrets of microsurgery are very helpfully revealed.

This book is well produced apart from a number of obvious misprints. Tartar writes concisely and has given us excellent measure. *The Biology of Stentor*, though a book for the specialist, is indispensable to every zoological, cytological and embryological library, and many professional cytologists and protozoologists will want to own it. J. A. KITCHING

BASIC APPROACHES TO STRUCTURAL ANALYSIS

Energy Principles in Applied Statics
By T. M. Charlton. Pp. vi+112. (London: Blackie and Son, Ltd., 1959.) 25*s*. net.

IN many institutions a student learns to determine the deflexion of nodal points in a pin-jointed framework and the forces in members of a statically indeterminate framework by use of Castigliano's theorems. There is no doubt that many students (and engineers) never realize that the solution is valid only when the load-deflexion relationship for the particular structure is linear. For most of the materials and proportions encountered in traditional practice, this assumption can be made with confidence and, frequently, the various design codes which an engineer must follow have implicitly made the initial step. With the development of new materials, and with the increasing awareness by the structural designer of the importance of ranges of mechanical behaviour outside those previously considered, it is desirable that the teaching schools, and the practising engineers, should look more closely at the fundamentals of the methods of analysis used.

During the past ten years, Mr. Charlton has been the author of a number of articles in the technical Press, in which he has treated several of these fundamental theorems of classical analysis. His recent book brings together much of that early work, and, by concentrating on the simplest plane pin-jointed systems, emphasizes the concepts involved in the basic equations of structural equilibrium and compatibility, and directs attention to the general applicability of approaches to analysis using complementary energy and the method of virtual work.

The differences between the various approaches may not be apparent in some cases to a superficial reader, and five of the seven chapters conclude with brief paragraphs which place each section in its proper position within the framework of the whole text. Many readers would prefer that these signposts occurred at the beginning rather than the end of the course, and at both, rather than either; but since the halting places are so well chosen the point becomes one of minor significance only.

Throughout the text the author speaks in terms of stiffness and flexibility coefficients and the final chapter is devoted mainly to the properties of these coefficients for linear systems. These concepts are becoming increasingly valuable with the introduction of the electronic computer in structural analysis, since the necessary equations may be set up in a systematized manner and lend themselves to solution by matrix methods on the machine.

The book is no vade-mecum for practising structural designers and does not purport to be. It would be, however, of great benefit to the good student and, one suspects, to most teachers of the theory of structures for, with both, fundamental principles are frequently obscured by an undue concentration on the techniques of analysis.

R. C. COATES

Pulp and Paper

Chemistry and Chemical Technology. By James P. Casey. Vol. 2: Papermaking. Second edition, revised and enlarged. Pp. xxiii+581–1249+147. (New York: Interscience Publishers, Inc.; London: Interscience Publishers, Ltd., 1960.) 188*s*.

THE scope of the second volume of this work is indicated in the review of Volume 1 (*Nature*, **190**, 380; 1961). Much the same general comments apply to Volume 2. Thus, British readers will be surprised at the absence from the chapter on fibre bonding of any reference to the important 1957 Cambridge symposium on that subject.

However, this should not be allowed to detract from a recognition of the general excellence and high standard of this work as a whole. It contains a tremendous amount of information of a fundamental nature covering paper-making operations from stock preparation to supercalendering, and taking in water usage and disposal, microbiological considerations and beater and wet-end additives.

Incidentally, it is completely self-contained in its subject-matter, so that there is no necessity to possess Volume 1 in order to derive the fullest benefit from Volume 2. This fact may appeal to many in Britain who are more interested in paper making than in pulping and to whom the cost of these books must appear very high.

JULIUS GRANT

Gas Chromatography Abstracts, 1958

Pp. vii+262. 42*s*.

Gas Chromatography Abstracts, 1959

Pp. ix+164. 42*s*.

Edited by C. E. H. Knapman, assisted by C. G. Scott. Sponsored by the Gas Chromatography Discussion Group associated with the Hydrocarbon Research Group of the Institute of Petroleum. (London: Butterworths Scientific Publications, 1960.)

THE two volumes of gas chromatography abstracts have been sponsored and prepared for publication by the Gas Chromatography Abstract Editorial Committee of the Gas Chromatography Discussion Group. The first is a combination of bibliographies compiled independently by three members of the Committee and covers the literature until the end of 1958. The 1,468 abstracts are listed alphabetically according to the first author. Considering the somewhat limited resources, reliance has had to be placed on abstracting journals particularly in connexion with foreign publications. This cannot be said of the second volume, which is compiled from abstracts obtained from 28 abstractors who between them have covered more than 110 journals. Although no precise date is given, the 717 abstracts in this volume appear to cover the literature until the end of 1959.

The abstracts in both volumes, although short (averaging 30–50 words), are very concise and to the point. Easy reference is made possible by author and subject indexes, the latter in particular being most comprehensive, enabling a complete survey of the literature on any topic to be carried out with great rapidity. Although the difficulties of compiling works of this type must be very great, few errors appear to have crept in. One criticism, however, is the frequent omission of any indication of the language of the article abstracted.

These volumes were compiled by the voluntary efforts of gas chromatographers, who, seeing a need for an efficient abstracting system, pooled their resources and produced their own. Their achievement deserves the highest praise and will certainly be welcomed by many wishing to keep abreast of this rapidly expanding field.

A. GOLDUP

South African Animal Life

Results of the Lund University Expedition in 1950–1951, Vol. 7. Edited by Bertil Hanstrom, Per Brinck, and Gustaf Rudebeck. Pp. 488. (Stockholm: Almqvist and Wiksell, 1960.) 75 Sw.kr.

VOLUME 7 of *South African Animal Life*, in which results of the Lund University Expedition (1950–51) are reported, maintains the high standards of the previous volumes in this series. Groups dealt with are preponderantly from among the Coleoptera, Diptera, Hymenoptera and Hemiptera. There are also chapters on the Oligochaeta, Decapoda, Crustacea and on the Isoptera. The authors are again recruited from many countries and seats of learning, and of the twenty-seven articles three are written in French, eight in German and sixteen in English.

O. LOWENSTEIN

A Collection of Tables and Nomograms for the Processing of Observations made on Artificial Earth Satellites

By I. D. Zhongolovich and V. M. Amelin. (Mathematical Tables, Vol. 12.) Issued by the Institute of Theoretical Astronomy, Academy of Sciences of the U.S.S.R. Pp. iv+188. (London and New York: Pergamon Press, 1961.) 100*s*. net.

THIS volume is a welcome addition to the very few existing mathematical tables which are designed to help in the calculation of artificial satellite orbits. The main table gives the true anomaly and the ratio of radial distance to semi-major axis, for a complete range of values of both mean anomaly (at 1° intervals) and eccentricity (from 0 to 0·76 at intervals of 0·01). Other tables give period and semi-major axis in terms of the mean angular motion, solutions of the equations used in calculating geocentric latitude and longitude, and the main secular perturbations of orbits. Finally, there are nomograms to determine the angle of elevation of a satellite. The intervals of tabulation and the number of figures in the tabulated quantities have been well chosen, and the layout of the tables is pleasing.

The tables themselves have been reproduced from the Russian original by a photographic process, and only the 10 introductory pages needed translating. Despite its minimal contribution to the subject-matter, the Pergamon Press has chosen to charge £5 for the volume, although it was advertised at 70*s*. This high price would be more justifiable if the 10 introductory pages were perfect, but they are not. There are irritating errors, such as the inconsistency of printing the symbol v as a Greek ν in the introduction but not in the tables. Also, the translation is at times sadly deficient. For example, the key sentence defining the units—"n is expressed in degrees per mean solar day and is given to 0·01 min. . ."—is nonsensical because of the omission (after 'day') of some 15 words, which give the tabulation interval for n and state that the orbital period is in minutes. These shortcomings do not, however, seriously mar the value of the volume to the specialist.

D. G. KING-HELE

STRUCTURE OF THE NORTHERN APENNINES

By Sir EDWARD BAILEY, F.R.S.
Formerly Director of H.M. Geological Survey
AND
Prof. W. J. McCALLIEN
University College of Ghana

IN 1957 we spent a full month in Florence, Spezia and Levanto, and have paid a few shorter visits since. In addition we have made an intensive study of publications, especially sheets of the Italian Geological Survey 1 : 100,000 map. We also have had the advantage of discussions with Prof. G. Merla and Dr. A. Valduga at the University of Florence; and we are specially grateful to the latter for conducting us over the exposures of the famous Carrara window. As a result of our inquiry we have come to conclusions that are in some respects novel. We consider that the rocks of the Northern Apennines, in large part Ligurian, can be conveniently assigned to four structural units as follows:

(1) The lowest unit is only seen in the Carrara window. Its rocks, as exposed, reach from Palæozoic, through Mesozoic to Tertiary, with a Miocene greywacke, termed Macigno, as their top member. The window interpretation advanced by S. Lencewicz[1] is now accepted by everyone.

(2) The next unit frames the window, and is widely, though intermittently, exposed in all directions therefrom. Its rocks, like those of unit 1, reach from Palæozoic through Mesozoic to Tertiary, with Miocene Macigno as top member.

(3) Above comes the Ligurian nappe, an old name, but used by us here in a somewhat extended sense. Its main rocks are a Jurassic submarine volcanic group, a Cretaceous shale group and an Eocene limestone, called Alberesi; and there is locally a further repetition of Macigno. The volcanic group, in addition to gabbro and ash, abounds in serpentinite, pillow-lavas and radiolarian chert, an assemblage which, here and elsewhere, has been called the Steinmann trinity. Detached masses of these rocks, sometimes of mountainous proportions, sometimes mere boulders, are distributed over an area measuring 200 km. by 100 km.; and everywhere they manifestly overlie Tertiary sediments, the Macigno of unit 2. They were for many years supposed to be themselves Tertiary, until Steinmann[2] demonstrated their Jurassic age. Ever since, they have ranked as particularly clear examples of older rocks transported for an amazing distance over younger rocks. Steinmann, coming from the complicated Pre-Alp nappe of Simmenthal in Switzerland, regarded the transport as a subterranean tectonic operation, a view with which we agree.

A difficulty concerns the remarkable discontinuity of the igneous outcrops. L. W. Collet[3], a specialist in nappe structures, attributed their separation in large measure to erosion, and has called them klippes, in the sense of the klippe outliers of the Lucerne district of the Alps. This is clearly a mistake, for the masses, large and small, lie already separate in a matrix furnished by the Cretaceous shale, itself broken to pieces. The whole constitutes a *mélange* in the sense E. Greenly has applied the term to the Gwna *Mélange* of Anglesey. C. I. Migliorini[4], followed by Merla and others, has gone on to interpret the Ligurian *Mélange* as a product of superficial stage-by-stage landslipping, supposed to have taken place from a series of anticlines, uplifted successfully farther and farther to the north-east.

A strong objection to this interpretation is, in our opinion, furnished by the restriction of the igneous enclosures to the shale outcrops, where they occur by the thousand. They are very seldom, if ever, found in the associated, far-travelled, and supposedly land-slipped Alberesi. Our view is that the *Mélange* is due to underground flow of the mobile shale material during nappe formation. Another argument against the superficial landslip interpretation follows from unit 3 showing a layered structure within itself, which

Fig. 1. Geological map showing four structural units in the Northern Apennines. Numbers in circles refer to map sheets of Italian Geological Survey 1 : 100,000. The other numbers are those of the structural units

can be traced through a succession of folds, persisting for many kilometres. Thus in the frame of the Carrara window the structural succession in unit 3 is Ligurian *Mélange*, on Alberesi, on Ligurian *Mélange*, on Macigno of unit 2; while on the two sides of the Spezia anticline (see Sheet 95 of the Italian map) Alberesi is poorly represented, and the structural sequence is, broadly speaking, Macigno, on Ligurian *Mélange*, on Macigno of unit 2.

(4) The top unit may be called the Genoa nappe, and consists of a great sheet of Alberesi, strikingly discordant to the contents of unit 3.

We hope shortly to furnish detail sufficient to establish the truth of the above outline sketch. Meanwhile, like P. Fallot, we may direct attention to a recent account by T. Rocco[5] of boring and geophysical exploration in the Po Valley in pursuit of oil. The Po Quaternary sometimes exceeds 2,000 m. in thickness, and the late-Tertiaries below it furnish another 8,000 m. These sediments are gently folded and are supposed to occupy the foredeep of the Apennines. If so, we suggest that the Po Valley is homologous to the Swiss Plain, and that the articulation of the Alps with the Apennines is homologous with the articulation of the Juras with the Alps.

[1] Lencewicz, S., *Estr. R. Soc. Sci. di Varsavia* (1917).
[2] Steinmann, G., *C.R. Fourteenth Intern. Cong.*, Madrid, 1926, **2**, 637 (1927).
[3] Collet, L. W., *The Structure of the Alps*, 266 (London, 1935).
[4] Migliorini, C. I., *Rep. Eighteenth Intern. Cong.*, London, 1948, **13**, 186 (1952).
[5] Rocco, T., *Proc. Fourth World Petroleum Cong.*, Rome, 1955, **1**, 675 (1956).

ORGANIC CONSTITUENTS OF METEORITES

By MICHAEL H. BRIGGS
Department of Chemistry, Victoria University of Wellington, New Zealand

PROF. J. D. BERNAL has recently commented[1] on the significance of carbonaceous meteorites in theories on the origins of terrestrial life. He points out that the reported[2] organic constituents of these meteorites may have played a part in the formation of life on Earth, or may be derived from some extra-terrestrial life-form. Alternatively, the meteorites could be fragments of Earth that broke away before the development of life, but after the abiotic formation of the complex compounds that are widely assumed to have given rise to the first terrestrial life-forms by a process of chemical evolution.

In this article I wish to discuss my own analytical results on a carbonaceous meteorite, and then to attempt to assess the available evidence concerning the origin and importance of meteorite organic matter.

Investigations of the organic constituents of meteorites have so far revealed materials of little biochemical interest. This could be due to the methods of extraction. Mueller, for example, removed compounds from the Cold Bokkeveldt meteorite with a variety of organic solvents, while Nagy *et al.* resorted to high-temperature distillation. Yet biochemicals are characteristically very soluble in water, and Calvin *et al.* have shown that some of the carbon in both the Orgueil and Murray meteorites is removable with water. With this in mind I have prepared hot-water extracts of a carbonaceous meteorite that fell at Mokoia, near Wanganui, New Zealand, in 1908.

The Mokoia meteorite consists mainly of two pieces (Fig. 1), with a total weight of approximately 10 lb. These two stones were found two days after impact. Consequently there is no chance that they picked up organic matter by prolonged contact with the soil. Since discovery the meteorite has been in the Wanganui Museum. The circumstances of discovery[3], together with the chondritic structure (Fig. 2), render the meteoritic nature of the stones unquestionable. Additional evidence is provided by the chemical composition (Table 1) and the X-ray diffraction analysis (Fig. 3).

Small samples of the meteorite have been supplied by the New Zealand Geological Survey. These fragments have been extracted by the following method:

The sample was first washed with distilled water and the upper 2 or 3 mm. removed from all over the surface by abrasion. In this manner the chances of extracting any surface organic compounds picked up by the stones from perspiration were minimized. The remainder of the sample was crushed to a fine powder by pestle and mortar, and the powder extracted with distilled water in a Soxhlet apparatus for two days. After filtration and evaporation of the solution so obtained, a pale brown residue was secured. This residue represented approximately 2 per cent by weight of the original meteorite fragment. The elemental composition of this residue varied quite appreciably from one meteorite fragment to the next. One residue contained approximately 6 per cent carbon, 1·3 per cent hydrogen, with traces of nitrogen. Spot tests carried out to detect inorganic constituents gave negative results for carbonate,

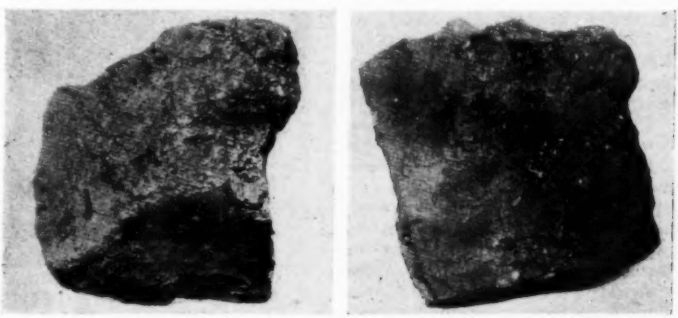

Fig. 1. The Mokoia meteorites. The two main pieces photographed soon after collection. Left, weighed 5 lb. 3 oz.; right, 5 lb. 2 oz.

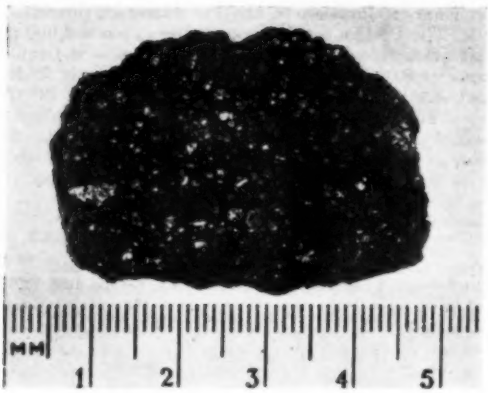

Fig. 2. Section of the Mokoia meteorite. Close-up of a small fraction (*c.* 40 mm. across) of the meteorite

bicarbonate, nitrate, nitrite and ammonium. It was therefore concluded that the residue contained water-soluble organic compounds, with some organic nitrogenous substance present in trace amounts.

Inorganic constituents actually detected by spot tests were the following: magnesium, sodium, potassium, sulphate, phosphate, arsenate and chromate. Negative results were obtained in tests for all other common inorganic anions and cations.

To identify the organic compounds present has so far proved an impossible task, due mainly to the very small quantities available for investigation. However, by the use of ultra-violet and infra-red spectroscopy, and of paper chromatography, some tentative conclusions have been drawn.

Small amounts of the residue have been submitted to paper chromatography in a variety of solvents. By the use of sprayed reagents several spots have been developed and their R_F values calculated. Of the many compounds detected in this manner, four of the most interesting will be described.

Compound I is strongly fluorescent in the short ultra-violet (below about 320 mμ) and may be detected on paper by this property. From the shape of the spots in several solvents it is possible that this is more than one substance. The descending R_F value at room temperature on Whatman No. 1 paper in water-saturated phenol is about 0·9. The compound also reacts very slowly on paper with picric acid to give a yellow-orange colour.

Compound II is also strongly fluorescent in short ultra-violet, but unlike compound I has a very low R_F in water-saturated phenol (less than 0·1). Nor does this spot react with picric acid.

The fluorescence of both these compounds is unaffected by *p*H. Both dissolve readily in ammonia, but not in hydrochloric acid.

Compound III may be detected on paper by the two tests normally used in the detection of purines on chromatograms. When sprayed with silver nitrate solution, then bromophenol blue, then washed with water, a very pale blue spot remains. When sprayed with silver nitrate, then potassium chromate solution, then washed in dilute nitric acid, a pale red spot remains that slowly fades. These two tests give a spot with the same R_F value in all solvents so far used. The spot cannot be detected on paper by examination in short ultra-violet, but if the chromatogram is first sprayed with a very dilute solution of fluorescein, the spot can be detected by its weak absorbance of ultra-violet. The R_F values of this compound on Whatman No. 1 paper at room temperature are: 0·1 *N* ammonium hydroxide (ascending) 0·87; 86 per cent *n*-butanol (descending) 0·23; *n*-butanol–water–acetic acid (descending) 0·13.

Compound IV was detected in only one meteorite fragment and not in two others. It was observed on paper chromatograms which had been allowed to run overnight so that the solvent (86 per cent *n*-butanol with 5 per cent by volume conc. ammonia solution) had run off the end of the paper. The compound consequently has a very low R_F in this solvent. The wet paper chromatograms showed no markings, but on allowing the papers to dry at room temperature a dark red spot appeared with a 'tailed' appearance. This experiment was successfully repeated several times on the residue from one fragment. The spot could be drawn down from the origin only by allowing the solvent to run for many hours. Acid solvents were without effect; only ammoniacal solvents were successful. On this basis it is possible that the red colour is produced by a reaction of some organic constituent of the meteorite residue reacting with magnesium hydroxide formed *in situ* by the basic solvent. Quite a variety of organic compounds will form red complexes with magnesium.

While it is impossible to identify any of these compounds from this information, tentative hypothe-

Table 1. Composition of a Fragment of the Mokoia Meteorite

	Per cent
Fe_3O_4	36·95
FeS	5·64
NiO	2·20
MnO	trace
SiO_2	37·55
Al_2O_3	2·62
CaO	3·50
MgO	6·30
P_2O_5	0·64
Na_2O	2·86
CoO	trace
C	1·25
Total	99·51

This analysis is by B. C. Aston and is reported by Marriner in ref. 3. Analyses on fragments used in the present work gave carbon values of 1·36 per cent.

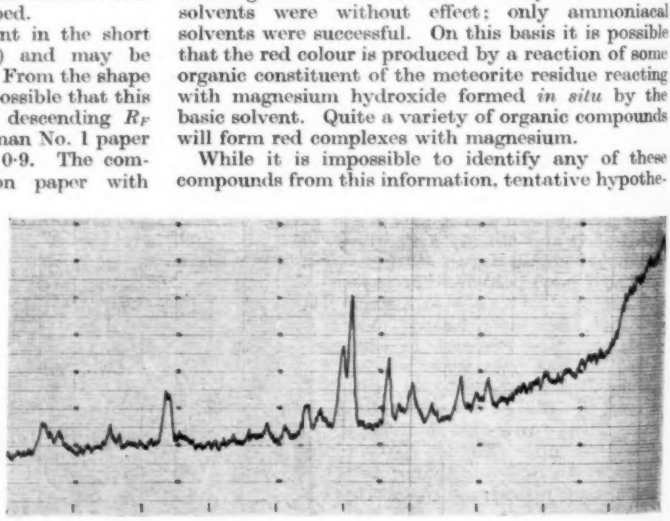

Fig. 3. X-ray diffraction analysis of the Mokoia meteorite. Determined on the powdered meteorite; wide slit, iron tube

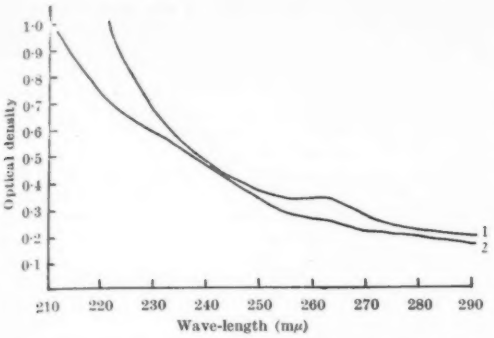

Fig. 4. Ultra-violet spectrum of meteorite extract. Curve 1 at *p*H 7; curve 2 at *p*H 2

ses concerning their constitution can be postulated. Thus from its reactions compound III must be able to form a silver compound capable of reacting with bromophenol blue or chromate to give a coloured complex. This immediately suggests a purine or related imidazole compound. Similarly, the fluorescent properties of compounds I and II suggest some conjugated ring system; while the water-solubility of all indicate the presence of polar groupings, such as carboxyl, hydroxide and amine. The low quantity of nitrogen in the residue indicates that amines can be present only in trace amounts.

Further information concerning the organic constituents of the residue is provided by ultra-violet and infra-red absorption spectra. In the ultra-violet the most characteristic property of the spectrum (Fig. 4) is a high end-absorption. However, the absorption curve varies somewhat with the *p*H of the solution. In neutral solutions there is a significant increase in absorption around 260 mμ as compared with the spectrum of acid solutions; suggesting an acidic chromophore.

Little information is given by the infra-red absorption spectrum of the intact residue; beyond a weak C—H absorption around 2,900 cm.$^{-1}$, a weak C=O absorption around 1,725 cm.$^{-1}$, and a strong double absorption at 860 and 880 cm.$^{-1}$. Most of the spectrum consists of strong, broad absorptions due to sulphate and water (even in carefully dried samples). One residue sample gave a sharp absorption at 1,395 cm.$^{-1}$; but this was not detected in other samples from different meteorite fragments. It is possibly significant that the residues giving this absorption also contained compound IV.

In an attempt to secure more information the residues were fractionated with different solvents. Cold water was found to dissolve only a part of the residue. After evaporation this cold-water-soluble portion was extracted first with chloroform, then with ethanol. All solvents used were carefully re-distilled analytical reagents that gave no residues on evaporation. The original hot-water-soluble extract of the meteorite was thus separated into four parts: cold-water-insoluble (residue *A*), chloroform-soluble (residue *B*), ethanol-soluble and chloroform-insoluble (residue *C*), and cold-water-soluble, ethanol-insoluble, and chloroform-insoluble (residue *D*). The infra-red absorption spectrum of each of these residues was determined, and the results are shown in Fig. 5.

Residue *A* shows few organic absorptions. There are a weak C=O and C—H absorptions, and absorption maxima at 2,500, 2,350, 880 and 800 that could be due to organic compounds. Residues *B* and *C* show the spectra with the greatest detail. Again C—H and C=O absorptions are present as well as a large variety of other peaks due to organic compounds. Residue *B* was not fluorescent. Residue *C* was strongly fluorescent and contained compounds I, II and III. After extraction with the two

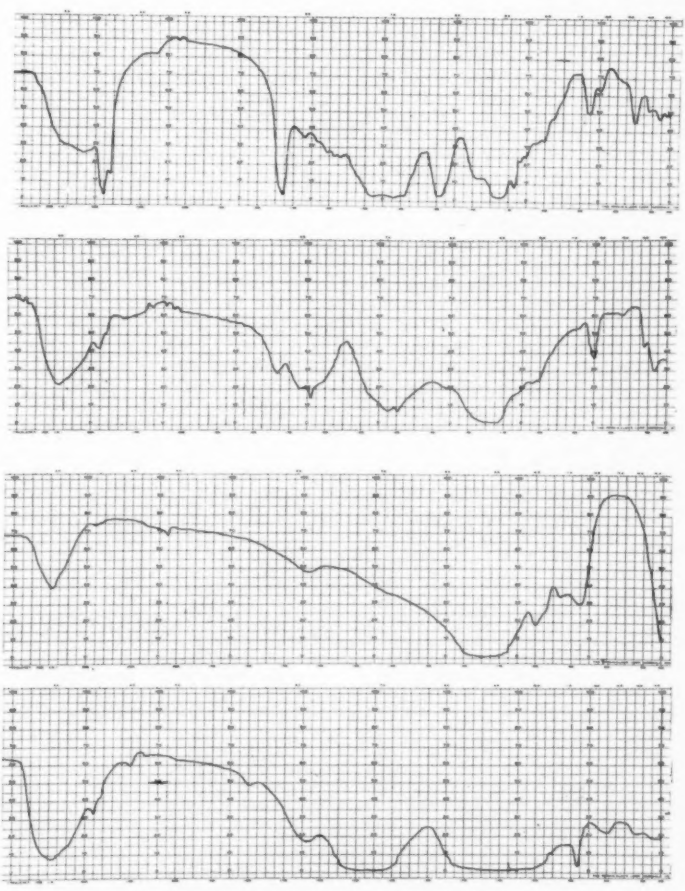

Fig. 5. Infra-red spectra of various fractions of the Mokoia meteorite extract. Spectra in descending order: chloroform-soluble fraction; ethanol-soluble, chloroform-insoluble; residue after chloroform and ethanol extraction; hot-water soluble, cold-water insoluble

organic solvents, residue *D* obviously contains little organic material, though a very weak C—H is present.

Residue *A*, which did not contain either compound I or II, was weakly fluorescent; indicating the presence of a third fluorescent compound.

Considering the evidence given here as a whole, it is apparent that the Mokoia meteorite contains a variety of quite complex organic compounds. Preliminary attempts to extract the carbon from the ground meteorite residues after water extraction with organic solvents have proved successful, and indicate the presence of water-insoluble organic material.

The problem raised by this, and other, work on meteorites is to provide a satisfactory explanation of the origin of these organic compounds. It is quite clear that, at least in the Mokoia case, the compounds are actual constituents of the meteorite and not terrestrial contaminants. The circumstances of discovery, preservation and the lack of such common soil compounds as amino-acids all render a contamination hypothesis untenable.

There would seem only two main possibilities for the origin of the meteorite compounds. Either they are the decomposition products of an extra-terrestrial life-form, or they are abiotic compounds formed early in the development of the solar system.

It has recently been demonstrated[4] that the cosmic-ray exposure ages of stony meteorites are all less than about 2×10^9 years, while the solidification ages are around $4{\cdot}5 \times 10^9$ years. This can be interpreted as evidence that the meteorites are fragments of a larger body that has disintegrated. While the cosmic-ray exposure ages of carbonaceous meteorites have not been determined, their overall similarity to non-carbonaceous chondrites makes the hypothesis that the two groups have cosmic-ray ages that are approximately the same at least plausible. Now the Earth has borne life for more than 2×10^9 years[5], hence, if carbonaceous meteorites have a cosmic-ray age less than this, it is unlikely that the organic compounds carried by these meteorites played any part in terrestrial biopoiesis.

However, several workers[6] have cast doubt on the interpretation of cosmic-ray ages, and propose that the meteorites and asteroids are not fragments of a larger body but have always been small objects. Several authors[7] also have suggested that the major features of Earth and Moon were produced by meteorite collisions. If this is true, it is evidence of the existence in the past of meteorites before the beginnings of terrestrial life.

The problem of the origin of the meteorite compounds is closely bound up with the larger problem of the origin of meteorites. If these bodies are fragments of a larger mass that no longer exists, then the compounds could be the remains of some exotic life-form. However, if the meteorites are not fragments of this nature, the compounds can only be the products of reactions of substances adsorbed on to surfaces. The numerous recent demonstrations[8] that highly complex compounds can be formed from simple materials by discharges and radiation suggest that organic syntheses could occur within a large cloud of meteorites in space.

Prof. J. Oro has recently suggested[9] that biochemical compounds would be formed at the point of impact of a comet with a planet. The asteroids, which obviously present a much larger target for comets, must have been in such collisions many times. Consequently the meteorite organic compounds could be the results of such collisions.

It is apparent that two major advancements remain to be made in this field. First, the chemical nature of the meteorite compounds must be established and mechanisms for their synthesis devised. Secondly, the cosmic-ray exposure ages of the carbonaceous chondrites must be measured to allow an estimate of the significance of these compounds in the early history of the solar system to be made.

I wish to thank Dr. W. A. Watters of the New Zealand Geological Survey for supplying samples of the Mokoia meteorite and for helpful advice; Dr. B. Cleverly and Dr. P. Grant of the Dominion Laboratory for the infra-red spectroscopy; Dr. N. F. Curtis and Mr. D. A. House for assistance with the inorganic spot tests; Dr. A. T. Wilson for assistance with the X-ray diffraction determination and for numerous helpful discussions; and Mr. D. Ward for helpful discussions on the infra-red analyses.

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THE WORLD UNIVERSITY SERVICE

By HILDA M. BROWNE
General Secretary

FOR three generations since 1920, the promotion of international co-operation and mutual service between and within universities and centres of higher learning throughout the World has been a task carried out by the organization known first as European Student Relief, then as International Student Service, and finally, since 1951, as World University Service. Its material aid programme has been complemented by the facilities it offers for the exchange of knowledge and experience gained in solving basic problems within its sphere. Non-partisan in its attitude, it makes no discrimination as to race, colour, religious belief or political creed.

The officers of the Co-operating Committee for Great Britain and Northern Ireland are: *president*, Sir Walter Moberly; *chairman*, Prof. W. R. Niblett; *hon. treasurer*, Sir Robert Mayer; *general secretary*, Miss Hilda M. Browne, World University Service, 59 Gloucester Place, London, W.1. The address of the International Headquarters is 13 Rue Calvin, Geneva.

XUM

The World University Service programme of aid, material and intellectual, is planned by mutual agreement among peers; need does not create inequality within the Service. The problems of a community, as they are seen by those who experience them, are presented for solution to the International Assembly, which consists of representatives, senior and student, drawn from the forty-two countries in which there are national World University Service committees. By accepting a project for inclusion in its programme, the Service can publicize the need and draw to it government and public interest and help.

In this way, health services have been established in countries where ill-health saps the student potential; hostels and student centres have been set up to improve living conditions; educational equipment and scholarships have been provided and funds created for the benefit of refugees, both student and teacher, in flight from man-made and natural disaster.

In Africa this year, the World University Service will assist community development projects on literacy, hygiene and construction which will bring together manual workers and members of the university community in a common effort and so restore the relationship with their own people of the small *élite* in the European-type university colleges. A five-year plan is being developed for the establishment of health services at university colleges in Basutoland, the Congo, Ethiopia, Kenya, Rhodesia and the Sudan, where the increasing influx of students will make medical examination even more essential in the future than it is now. Pius XII College, Basutoland, the only non-segregated college in South Africa, is being besieged by applicants who must be rejected for lack of space. The World University Service is to help with a vast project to raise sufficient funds for building a new Department of Education for which the organization will provide furniture and equipment, and aid will be given to a co-operatively run bookstore supplying text-books and materials not easily obtainable from the usual commercial sources. University College, Ibadan, Nigeria, is to open a co-operative hostel, to relieve the shortage of accommodation which is limiting acceptances. Medical equipment will be supplied for the student health service at the University of Khartoum, and financial help given towards a co-operative book-store there also. For many years the Service has given aid to the African Medical Scholarships Trust Fund, which enabled non-European students to study medicine at the University of the Witwatersrand, and latterly to the Non-European Students Fund which helps students at the University of Cape Town. The passage of the Extension of University Act, limiting the African Medical Scholarships Trust Fund and the Non-European Students Fund to continued support of students already enrolled at the two Universities, was followed by the establishment of the South African Committee for Higher Education, the funds of which are enabling non-Europeans to work externally for university degrees. The World University Service International South African Student Fund is supporting the South African Committee for Higher Education, making supplementary awards to students at Cape Town and providing emergency assistance to university personnel suffering through apartheid.

Direct health projects in Asia, where so much has already been done as the consequence of a student health conference held in 1951, include the establishment of the first health centre at the Chinese Language Colleges in Hong Kong, of student wards at the Banares Hindu University and the University of Bombay, of a comprehensive health service at the latter University, of out-patient care for tubercular students in Japan, where the Service already has two sanatoria, and of a clinic at Seoul, Korea. In addition, medical equipment and supplies, and X-ray apparatus, will be sent to Pakistan and Vietnam, and financial support given for the expansion of the student tuberculosis ward at Masan, Korea, and for further development of the anti-tuberculosis campaign in Thailand.

Outside the field of medicine, other preventive measures must be taken in Asia to combat the danger of ill-health arising out of poverty and malnutrition. Co-operative projects, which provide living accommodation, food, books, stationery, etc., at prices well below the commercial level, can do much to lessen the cost of living for the student. Following a conference on co-operative techniques organized in January 1959 in Madras at which experts addressed the representatives of ten south-east Asian countries, co-operative cafeteria, hostels, student centres, libraries, book-stores, book banks and employment bureaux have been set up by World University Service committees in Burma, India, Indonesia, Japan, Nepal and Pakistan.

Since the end of the Second World War, one of the main duties of European World University Service committees, including that of Great Britain, has been the care of refugee students and staff members in their countries. The two major crises in the past few years have been primarily the Hungarian uprising, when more than 7,000 staff members and students fled to Austria and Yugoslavia, and the flight of Algerian students from France and their native country into Europe, Tunisia and Morocco. Both called for strenuous efforts by the Service to raise funds on the local level and from trusts and foundations in order that staff members might gain the necessary qualifications for continuation of their work in the country of their adoption and that students might have the opportunity of completing their interrupted studies. In Great Britain, the Hungarian refugee settlement programme has provided free scholarships for university study for 119 students, and supplementary grants for those studying at technical and other colleges of higher education. More than £36,000 has been spent by the Service on scholarships and material for Algerian refugee students, many of whom are still in dire need.

Gradually, from the narrow front of Vienna in 1920, the field of activity has spread and moved, as the need for the World University Service help has declined in one area and grown in another. The demand for help always exceeds the supply of funds, which, apart from those drawn from major foundations and non-government agencies for very large-scale special projects, are derived from the community most concerned with higher education.

In Great Britain it is agreed that education should be given according to the capacity of the individual: we must also face our responsibility for the practice of this creed in the emerging and less-prosperous lands, particularly those having direct links with our own. Until they approach our level of economy, World University Service can do much to remove the barriers to teaching and learning set up by poverty, ill-health and lack of equipment.

XUM

OBITUARY

Dr. G. C. J. Dalton

It was with deep regret that atomic energy scientists throughout the world learnt of the death of Dr. G. C. J. Dalton on July 17.

Dr. Dalton was born in Te Awamatu in the centre of the North Island of New Zealand just a little more than forty-five years ago. After a distinguished university career at Auckland and Canterbury University Colleges, Dalton was awarded a Rhodes Scholarship in 1936. At about this time he characteristically played in a major rugby football match while suffering from influenza; in his subsequent weakened condition he contracted poliomyelitis which resulted, after a year's illness, in the loss of the use of one of his legs. This did not deter him, and his strong traits of courage, fortitude and determination enabled him to overcome this physical disability. It did not prevent him from breaking his studies at Oxford to take an active part with the Royal Air Force in the radar defence of Britain, to enjoy rowing at Oxford, tennis at Harwell and swimming in Sydney. These brave and unselfish characteristics again revealed themselves during his fatal illness when he continued his work long beyond the time when he could humanly or medically be expected to do so.

Dalton claimed that as the engineer had to design equipment to operate, "he had to know his physics better than the physicist". Dalton himself certainly showed a penetrating and critical approach to the basic scientific problems, and his mastery of these, coupled with his considerable mathematical ability, enabled him to make major contributions to both research and teaching in the newer engineering developments, such as atomic energy.

These broad scientific interests coupled with a critical and imaginative mind showed themselves to full advantage in his initiation of the fast reactor programme at Harwell, in academic life as dean of the Faculty of Engineering in the University of Auckland, in the formulation of the Australian high-temperature gas-cooled reactor programme, and from 1960 as director of the Australian Atomic Energy Research Establishment.

To quote Sir Mark Oliphant: "Dr. Dalton was a warm friend of all who thought about the new ideas in engineering, helpful and generous of his time and effort, inspiring to young and old, and completely devoted to his work, his family and his ideals".

His loss is indeed a grievous one to Australia, to atomic energy and to his many friends and colleagues scattered throughout the Western world.

C. N. Watson-Munro

NEWS and VIEWS

New Appointments at Associated Electrical Industries, Ltd. : Mr. L. J. Davies, C.B.E.

Mr. L. J. Davies has recently been made responsible for the direct supervision of the four Research Establishments of Associated Electrical Industries, Ltd., at Aldermaston, Harlow, Manchester and Rugby. Mr. Davies was educated at Oxford, going there with an open exhibition in natural science, taking his first degree and carrying out postgraduate work, joining British Thomson-Houston Co., Ltd., in 1924. For the past sixteen years he has been director of research at Associated Electrical Industries (Rugby), Ltd. During this period, under his guidance, many changes have been made in the scope and nature of the work and in the laboratory buildings, culminating in the setting up and occupation last year of completely new buildings on an eight-acre site reserved for research. For a period his interests were concerned with lamps and lighting. He was closely connected with the introduction into Britain of the fluorescent lamp and in a form that made it suitable for production for factory lighting during the Second World War. He was awarded in 1942 (with co-authors) the Crompton Premium of the Institution of Electrical Engineers for a paper on this subject. He is a past president of the Illuminating Engineering Society, a member of Council of the British Electrical and Allied Industries Research Association, and chairman of the Board of Governors of Rugby College of Engineering Technology. He was twice Faraday lecturer of the Institution of Electrical Engineers, and was made C.B.E. in 1960.

Dr. J. E. Stanworth

Dr. J. E. Stanworth has been appointed as director of research of Associated Electrical Industries, Ltd., in succession to Mr. L. J. Davies. Dr. Stanworth is a native of Lancashire, and was initially educated at Nelson Secondary School, from which he went to the University of Sheffield to gain a B.Sc. (Tech.) degree with honours in 1935 and his M.Sc. (Tech.) degree in 1937. He joined the British Thomson-Houston Co. as glass technologist in 1937. He spent his first years at Rugby in research on glasses and on glass structure, eventually leading a team on this subject partly in the research laboratory and partly in the glass works of the Company. He then took over wider duties involving the supervision of the research of a large number of groups engaged in investigating new materials and their applications, particularly in the fields of semiconductors, materials for nuclear power reactors, lamps, high-temperature materials and hard materials. Dr. Stanworth was awarded the degree of D.Sc.(Tech.) by Sheffield in 1958. He is a Fellow of the Royal Institute of Chemistry, the Institute of Physics and the Physical Society, and the Society of Glass Technology.

Medical Research Council: New Members

Prof. M. L. Rosenheim (professor of medicine in the University of London at University College Hospital Medical School) and Prof. Wilson Smith (lately professor of bacteriology in the University of London

at University College Hospital Medical School) have been appointed members of the Medical Research Council from October 1, 1961.

New Research Group

THE Medical Research Council has recently agreed to establish a Research Group at the University of Durham on demyelinating diseases. The Group, to be situated in the William Leech Research Laboratories, Royal Victoria Infirmary, Newcastle upon Tyne, will be under the direction of Dr. E. J. Field, senior lecturer in neuropathology, King's College Medical School, and consultant neuropathologist at the Infirmary; Dr. H. G. Miller, physician in neurology to the Infirmary, will act in an advisory capacity on the clinical side.

Feldberg Foundation for Experimental Medical Research

A FOUNDATION to endow an Anglo-German lectureship has recently been established by Prof. W. Feldberg, a senior member of the Medical Research Council's scientific staff at the National Institute for Medical Research. Prof. Feldberg has set up this Foundation out of funds awarded to him by the Federal German authorities as compensation for professional damages. It will be known as the Feldberg Foundation for German–English Scientific Exchange for Experimental Medical Research and will be administered by a Board of Directors consisting of three German and three British scientists. Each year the Board will award a prize to one German and one British research worker of distinction, and in recognition of this gift the German recipient will give a lecture in Britain and the British recipient a lecture in Germany. The prizewinners for 1961 are Sir Lindor Brown, Waynflete professor of physiology in the University of Oxford, and Prof. Herbert Hensel, director of the Physiological Institute of the University of Marburg. In November of this year, Prof. Hensel will lecture at University College, London, on "The Electrophysiology of Thermoreceptors", and Sir Lindor Brown will lecture at Heidelberg on "The Adrenergic Neurone". The German Federal Republic recently conferred a decoration, the 'Grosses Verdienstkreuz', on Prof. Feldberg in recognition of this generous endowment.

Change of Address

THE Medical Research Council's headquarters office will shortly be moving to new accommodation, and from September 18 its address will be: 20 Park Crescent, Regent's Park, London, W.1. Telephone: MUSeum 5422.

The International Atomic Energy Agency

THE International Atomic Energy Agency, Vienna, has issued a short pamphlet explaining the purpose which the Agency serves and describing its origin, structure and growth and what it has achieved since it came into being in October 1957. By April 1961 more than 600 senior workers from 50 countries had completed their training or were receiving it in 26 countries under the Agency's programme of nuclear science fellowships. The exchange and diffusion of information is another major programme under which four large conferences were held in 1959 and 1960, while under its technical assistance schemes, early in 1961, 30 Agency experts were serving in 16 countries and 7 composite teams had visited 31 countries. Another statutory function is to arrange for supplies of nuclear fuel at the request of member States, and the Agency has also formulated standards and measures of safety for various types of nuclear operations and has worked to establish internationally agreed legal obligations to compensate for damage caused by nuclear accidents. Besides research in its own laboratories on the preparation of standards of radioactive sources, calibration of nuclear equipment and analysis and handling of nuclear materials, the Agency sponsors research projects in member States.

Nuclear Energy Training Courses

As part of its expanding training programme, the International Atomic Energy Agency is organizing, in co-operation with some Member States and specialized agencies of the United Nations, four courses on various practical applications of nuclear energy during September 1961–February 1962. The first such course, on the "Use of Radioisotope Techniques in Soil–Plant Aspects of Agricultural and Forestry Research", held by the Agency jointly with the Food and Agriculture Organization and in co-operation with the Government of the Netherlands, at Wageningen, began on September 4 and will last until October 27. The course is under the direction of Prof. A. C. Schuffelen, head of the Laboratory of Soils and Fertilizers, Agricultural University, Wageningen, Netherlands, and Dr. C. H. Fuller, Brookhaven National Laboratories. A four-month advanced international training course on "Biological Effects of Radiation", commencing October 24, will be conducted by the Agency and the Government of Israel at the Radioisotope Training Centre near Rehovoth. Dr. Peter Alexander, of the Royal Cancer Hospital, London, United Kingdom, will be scientific adviser for this course. A four-week international training course on "Radiation Health and Safety", organized by the Agency and the World Health Organization in co-operation with the Government of Japan, will be held in Chiba City, Japan, during October 24–November 21. This course will be directed by Dr. J. N. Stannard, University of Rochester. A regional training course in the "Medical Applications of Radioisotopes" will be held by the Agency in co-operation with the Government of the United Arab Republic during November 4–December 28 in Cairo. Dr. William J. MacIntyre, University Hospitals of Cleveland, will be general adviser to the course. The courses in Israel and the United Arab Republic are financed from funds provided under the United Nations expanded programme of technical assistance. The sponsoring organizations will provide fellowships and some scientific equipment for the four courses. Further information can be obtained from the headquarters of the International Atomic Energy Agency, Vienna.

Glass in the Electronics Industry

IN many ways the lamp and vacuum tube industry is peculiarly dependent on glass as a raw material. Until the advent of semiconductors it was probably true to say that every lamp and electronic device contained several glass-to-metal seals, and the new vapour lamps, particularly the sodium and mercury vapour lamps, only became possible after the development of suitable new glasses to contain the metal vapours. It is appropriate, therefore, that the Philips organization should have devoted a double number

of the *Philips Technical Review* to the material glass (22, Nos. 9 and 10, 281; 1960–61). There is a most interesting introductory paper on the history of glass, which is beautifully illustrated with colour reproductions of ancient pictures showing the use of glass and monochrome illustrations of ancient pieces of glass, including one of a Roman cage cup which consists of a series of circles of glass joined together but which has been carved out of the solid. Of particular note are two carefully written articles, one describing the development of automatic bulb-blowing machinery and the other dealing with the automatic control of modern glass-melting tank furnaces. There is a brief reference to the Glass Development Centre opened at Eindhoven in 1957; this is a well-designed laboratory which has its own pilot plant capable of taking to the manufacturing stage any new glasses and articles made therefrom. The more fundamental investigations of glass which are carried out in the central research laboratories are represented by a characteristic article on the structure of glass from J. M. Stevels.

Ferro-Alloy Industry in India

Since the turn of the last century, technological developments in the production of ferro-alloys have been remarkable both in range and quality. The National Metallurgical Laboratory, Council of Scientific and Industrial Research, India, has embarked on some major research and development projects to investigate the suitability of Indian raw materials for production of a range of ferro-alloys to exacting specifications and to study the economical application of the latest metallurgical techniques.

A symposium on the ferro-alloy industry in India will be held at the National Metallurgical Laboratory, Jamshedpur, during February 1962. The symposium will cover the following: (1) survey of raw materials in regard to quality and quantity for production of different types and grades of ferro-alloys in India, including auxiliary requirements such as fluxes, fuels, electric power, transport, etc.; (2) beneficiation of raw materials for indigenous ferro-alloy industry based on physical, chemical and thermal beneficiation techniques and ore-dressing cycles, formulation of optimum flow-sheets and assessment of recovery yields and economics; (3) evaluation of electro-thermal, pyrometallurgical techniques and other specific processes and developments in the production of different types and grades of ferro-alloys in relation to possibilities for application in India in the context of indigenous resources; (4) study of the basic physico-chemical and thermodynamic principles involved in the production of ferro-alloys, including theoretical investigations on slag and metal equilibria and related fundamental hypotheses; (5) latest advances in technology of production of ferro-alloys considered in relation to the laboratory-scale, pilot-plant and industrial prototype production trials; (6) utilization of by-products obtained in ferro-alloy industries and overall economics of integrated production cycles; (7) standard specifications for different grades of indigenous ferro-alloys based on corresponding specification of the raw materials needed; (8) present status of ferro-alloy industry in India and future expansion plans to be projected during India's successive Five Year Plans in the general background of trends in world production. Invitations are being extended to technologists, metallurgists and research scientists in India and abroad to attend the symposium and contribute technical papers for discussion. The symposium will provide an international forum for establishing personal contacts and facilitating exchange of ideas on the development of ferro-alloy industry in general and that of India in particular.

Sub-bituminous Coals as Oil-well Drilling Mud-thinners

Modern procedure in drilling oil-wells, especially the rotary system, is to employ a heavy 'mud' fluid, for example, bentonite, to counteract hole-pressure, act as drilling-bit lubricant, assist in removal of rock-cuttings, and to seal borehole walls. It is customary to introduce additives or thinners to control the rheological properties of drilling mud, which normally tends to a progressive contamination, hence thickening, by rock-powder derived from cuttings. Such additives may be of chemical origin, but also natural humic acid types are employed (in the United States under the common but perhaps somewhat misleading name of 'lignite'). The active components of this type occur in naturally weathered or artificially oxidized coals, from which they can be extracted with aqueous solution. Several Alberta coals are rich in humic acids, and in a recent publication of the Research Council of Alberta, *Alberta Subbituminous Coals as Drilling Fluid Thinners*, by E. J. Jensen (Information Series No. 33. Pp. 25. Edmonton: Research Council of Alberta, 1961), it is concluded that deposits of suitable coals requiring no treatment but pulverization occur abundantly in this Province; that they possess good thinning qualities and are characterized by high cation-exchange capacity and fixed carbon : volatile matter ratio of less than unity. The amount of coal required for maximum thinning is 2–4 per cent by weight of the drilling mud. Further investigation by the Research Council into this interesting development is foreshadowed in this publication, which also includes relevant analytical and experimental data on these Alberta coals as potential sources of drilling mud thinners.

The Le Play Society

Sir John Russell's presidential address to the concluding conference of the Le Play Society at Wadham College, Oxford (April 9–13), has now been published by the trustees of the Frederick Soddy Trust (Pp. 22. Hove, Sussex: The Frederick Soddy Trust, 9 The Drive, 1961). After reviewing briefly P. G. F. Le Play's methods of studying a country and its people and the developments due to Patrick Geddes, Sir John describes the characteristic activities of the Society, including its summer journeys abroad, its classes on the technique of surveying and field surveys by groups of students and the annual conferences. The changed conditions after 1945 limited considerably the character of its post-war visits, quite apart from the great increase in costs, and Sir John also discusses in some detail the drastic changes in environment that modern technology has made possible, for example, in the Zuider Zee and elsewhere in Holland, and in Jutland and the Channel Isles. While the Society can look back with satisfaction on the part it has played in fostering and stimulating interest in survey work, especially the interactions of place, work, and folk, the work has grown beyond its powers, although both name and work will live through the Frederick Soddy Trust. Sir John concludes with a warm tribute to the work of Miss Margaret and Miss Alice Tatton.

XUM

Research into Problems of Ageing

THE second compilation of reviews of selected papers reporting research in ageing, supported by grants from the United States National Institutes of Health or conducted in the Institutes' laboratories, has been made from reviews of more than 500 papers published during the year 1960. These articles range from fundamental work on the biology of ageing to studies concerned with the physical, psychological, and social problems of ageing people. The number of grants made to medical schools, research hospitals, and other research institutions for investigations on problems of ageing has been mounting steadily. To-day, 16 million dollars of support is being provided by the National Institutes of Health for more than 700 projects in ageing. The compilation, entitled *Research Highlights in Ageing*, can be obtained from the Superintendent of Documents, U.S. Government Printing Office, Washington, 75, D.C., price 25 cents. (United States Department of Health, Education, and Welfare: Public Health Service Publication No. 854. Pp. vii + 50. 1961.)

The Plastics Institute

MR. L. M. READ, administration and services manager, Research and Development Laboratories, DCL Plastics Group, has recently been appointed chairman of the Council of the Plastics Institute. Mr. Maldwyn Jones, development director of the Plastics Division of Imperial Chemical Industries, Ltd., has been appointed vice-chairman. A Building Sub-Committee to deal with all matters consistent with the objects of the Institute relating to the development of the use of plastics in building has been set up. The following are members: H. D. Atkin (Allied Structural Plastics, Ltd.), G. A. Britton (John Laing Research and Development, Ltd.), Dr. W. B. Brown (Monsanto Chemicals, Ltd.), Dr. W. Dawson (Imperial Chemical Industries, Ltd., Plastics Division), D. Gonda (EPS (Research and Development), Ltd.), D. B. Honeyborne (Building Research Station), D. S. Mahon (Bakelite, Ltd.), Dr. Z. S. Makowski (Imperial College of Science and Technology), H. Stanley Smith (Richard Costain, Ltd.), K. Turner (chartered architect), V. H. Wentworth (Monsanto Chemicals, Ltd.).

The Night Sky in October

NEW moon occurs on Oct. 9d. 18h. 53m. U.T. and full moon on Oct. 23d. 21h. 31m. The following conjunctions with the Moon take place: Oct. 5d. 21h., Regulus 0·2° N.; Oct. 7d. 08h., Venus 0·5° S.; Oct. 17d. 05h., Saturn 3° S.; Oct. 17d. 14h., Jupiter 3° S.; Oct. 26d. 17h., Aldebaran 0·6° S. In addition to these conjunctions with the Moon, Mercury is in conjunction with Mars on Oct. 11d. 20h., Mercury being 4° S. Mercury is too close to the Sun for observation. Venus is a morning star, rising at 3h. 15m., 3h. 55m. and 4h. 45m. on October 1, 15 and 31, respectively. Its stellar magnitude on October 15 is − 3·4, the visible portion of the apparent disk increases during the month from 0·870 to 0·934, and its distance from the Earth increases from 131 to 144 million miles. Mars is too close to the Sun for observation. Jupiter is an evening star, setting at 23h. 25m., 22h. 35m. and 21h. 40m. on October 1, 15 and 31, respectively. It is near the border of Sagittarius and Capricornus; its stellar magnitude is − 2·0 on October 15 and its distance from the Earth is 450 million miles. Saturn is an evening star, setting at 23h. 05m., 22h. 10m. and 21h. 10m. at the beginning, middle and end of the month, respectively. It is in Sagittarius; its stellar magnitude is + 0·7 on October 15 and its distance from the Earth is 920 million miles. Occultations of stars brighter than magnitude 6 are as follows, observations being made at Greenwich: Oct. 20d. 21h. 55·5m., χ Aqr. (*D*); Oct. 27d. 2h. 40·1m., 318*B*. Tau. (*R*). There is also an occultation of Venus: Oct. 7d. 6h. 31·0m., (*D*); Oct. 7d. 7h. 56·1m., (*R*). *D* and *R* refer to disappearance and reappearance, respectively. The Orionid meteors are active during October 15–25, maximum activity being on October 20. The radiant is at R.A. 6h. 24m., Dec. + 15°, and conditions for observation are moderately favourable.

Announcements

DR. G. G. MACFARLANE, at present deputy director of the National Physical Laboratory (see *Nature*, **184**, 1844; 1959), has been appointed director of the Royal Radar Establishment at Malvern, in succession to Mr. W. J. Richards.

As from August 30, the address of the headquarters of the Department of Scientific and Industrial Research became State House, High Holborn, London, W.C.1. Telephone: Chancery 1262.

A CONFERENCE of professors of mathematics from the universities of Great Britain is being convened by the Committee of Vice-Chancellors and Principals of the Universities of the United Kingdom at the end of September to consider the teaching of mathematics. The proceedings will be private.

THE Royal Entomological Society of London is organizing a symposium on "Insect Polymorphism", to be held at the Imperial College of Science and Technology, South Kensington, during September 21–22. Further information can be obtained from the Registrar, Royal Entomological Society of London, 41 Queen's Gate, London, S.W.7.

THE second summer research institute of the Australian Mathematical Society will be held at the Australian National University, Canberra, during January 3–February 1, 1962. Analysis and number theory will be the main fields of research, and Prof. A. Erdelyi (California) will be the guest lecturer. Further information can be obtained from the director of the Institute, Prof. E. S. Barnes, Department of Mathematics, University of Adelaide, Adelaide, South Australia.

A SYMPOSIUM on "Optical Character Recognition" will be held during January 15–17, 1962. It will be jointly sponsored by the Information Systems Branch of the Office of Naval Research Information Centre of the National Bureau of Standards, and will be held in the Department of the Interior Auditorium on C Street, between 18th and 19th Street, N.W., Washington, D.C. Further information can be obtained from Miss Josephine Leno, Code 430A, Office of Naval Research, Washington 25, D.C.

ERRATUM. The article entitled "Reactions of Salt Solutions in Calcium Chloride on Mortar and Concrete", appearing on p. 125 of the July 8 issue of *Nature*, refers to two original articles appearing in the *Australian Journal of Applied Science* (**11**, No. 4, December 1960).

FROM RADAR TO SPECTROSCOPY: NEW REGIONS OF THE SPECTRUM*

By Prof. D. J. E. INGRAM

Department of Physics, University College of North Staffordshire, Keele

THE applied research that was concentrated on radar and microwave electronics during the War has now produced a new branch of research in pure science; it has opened what we might call a new 'window' in the spectrum of the electromagnetic radiations which stretch from the very energetic γ-rays at one end to the long wave-length radio waves at the other. This is one of the most striking examples of how development in applied physics can sometimes initiate a major advance in fundamental research and thus reverse the normal trend in scientific development.

The study of the emission and absorption of various types of radiation in the different regions of the electromagnetic spectrum may be taken as a reflexion of the progress of the whole history of physics, from its initiation with the classic experiment of Newton in which he split white light into its component colours. The detailed measurements on the spectral lines of different atoms in the visible region, followed by the beautifully simple explanation of all these complex frequencies by Niels Bohr in terms of quantitized orbiting electrons, are other major steps along this pathway. Somewhat earlier, too, the work of Madame Curie and others had shown that the nucleus itself would give off radiations of very short wave-length, and a study of these γ-rays has proceeded ever since to obtain as much information as possible about the nuclear forces themselves.

The electromagnetic spectrum can still be taken as the background to the advances in physics of recent years, and it can be said in all fairness that the two fields in which physics has made its greatest advances since the War have been, first, that of high-energy nuclear physics, with its study of radiations of very high energy produced by large accelerating machines; and secondly, radiophysics—or the study of the discrete radiations absorbed and emitted in the long wave-length region of the spectrum.

Radio astronomers have made particular use of this latter region of the spectrum; they have followed one discrete emission line in their investigations, as well as pursuing their general study of the radio noise, spread evenly across the spectrum, which can be detected from the remoter galaxies near the edge of the observable universe. This discrete spectral line of 21-cm. wave-length comes from the atomic hydrogen present in the arms of our own and other galaxies; and it is used by the radio astronomers to map the features of our own galactic system, and also to measure its speed of rotation from the slight changes that occur in the wave-length.

The terrestrial physicist is also very interested in this spectral line, although he uses it for exactly the opposite purpose: instead of following it out into the nearly infinite depths of space like the radio astronomer, he can pursue its origin back into the very heart of the atom, and deduce the nature and magnitude of the forces that are interacting there. It is possible to do this because the frequency, or wave-length, of the radiation emitted by an atom is related directly to the changes of energy which take place in it when the radiation is emitted. It follows that a careful study of the wave-lengths of these different radiations will therefore give very precise information on the energy-states existing within the molecules and atoms themselves, and, from this, on the nature of the actual forces which hold the atoms together.

The great advantage of carrying out spectroscopy in the radar or micro-wave regions is that very well-defined and highly stabilized wave-lengths are available, and these can be measured to accuracies of better than one part in a thousand million. An immense wealth of information is therefore available from the fine and hyperfine patterns that arise on these spectra, and which are produced by interactions with the nuclei of the different atoms. This was discovered very soon after the end of the War, when Bleaney and his co-workers in Britain, and Good in the United States, first applied the war-time radar techniques to a study of ammonia and other molecules.

Another branch of microwave spectroscopy, which was somewhat slower in starting than the work on gases, but which has now outstripped it, and developed widely in a variety of different directions, is that of electron resonance. In this type of experiment a magnetic field is applied across the specimen at the same time as it is bathed with the microwave radiation, and the energy jumps of the unpaired electrons can then be followed very precisely.

Some of the more practically minded might well ask at this point what relevance such investigations have to everyday life. One can usually justify most scientific investigations on two grounds: first, from an innate sense of curiosity, the desire to find out and unravel the truth so far as we can go; and secondly, from the fact that very useful and practical applications usually derive from this 'pursuit of truth' on the way. So it is with microwave spectroscopy. The war-time developments in radar opened up a vast unexplored region of the electromagnetic spectrum, and we have been eagerly and enthusiastically exploring all its features. This has afforded some new ideas and results which have proved to be of immense practical value.

First, the measurement of frequency and time—we find that the vibrations of the atoms are more regular and reliable than the rotation of the Earth and movement of the stars, and our standard time signals are now, in fact, controlled by microwave frequencies linked to the oscillations of cæsium atoms. A second application of increasing importance is the use of electron resonance as a probe for unpaired

* Substance of the Kelvin Lecture delivered on August 31 at the Norwich meeting of the British Association.

electrons in all kinds of systems—those formed by transient chemical reactions, those produced by irradiation damage, and, more recently, in biological systems involving cancerous growth or other abnormal metabolic activity.

One final example will serve to turn the wheel full circle and show how the latest results of the pure physicists in this field have now produced a new device which promises to be of immense help to the microwave engineer. This is the maser, which uses the atoms themselves to produce microwave amplification and oscillation, rather than any radio circuits or electron beams. Thus the debt to the applied scientist has been repaid, and this new line of research continues to develop and flourish as the different sides go on together.

BRITISH WELDING RESEARCH ASSOCIATION: NEW LABORATORY

THE official opening by Lord Mills of a new laboratory block of the British Welding Research Association at Abington Hall, Cambridge, on July 12, marked an important stage in the development of the Association's Research Station. Since 1946, when Abington Hall was first taken over by the Association, much of the research work has had to be carried on in some of the original outbuildings of the estate and in wooden huts remaining from war-time military occupation. The first new laboratory was built during 1951–52, to accommodate large-scale equipment for fatigue researches, and this was followed in later years by the construction of a second research block which permitted the integration at Abington of the metallurgical work previously housed in London. The present scheme represents the completion of a further stage that was put in hand three years ago with the building of a welding construction shop that now forms part of the new laboratory opened by Lord Mills.

The new block is a two-storey building, the steel frame of which, in common with the earlier laboratories of the Association, is of welded construction and designed by the plastic theory. The ground-floor, of 15,000-sq. ft. area, accommodates research sections working on the design of pressure vessels and pipe-lines, the brittle fracture behaviour of steel, and the development and application of resistance welding methods. It also contains a machine shop. Office and conference rooms are provided on an upper floor of 8,200 sq. ft. area. The laboratory was designed by Messrs. H. C. Hughes and P. Bicknell, of Cambridge, and erected by Messrs. Rattee and Kett, Ltd., also of Cambridge.

It is appropriate to recall that the pressure, brittle fracture and resistance welding investigations were among the first projects to be inaugurated at Abington. Each, therefore, has a fairly extensive background of development and, clearly, each is seen to retain an important future in the welding research programme as a whole.

The subject of the design of welded pressure vessels was recently discussed at a symposium organized by the Institution of Mechanical Engineers in collaboration with the Association. With a theme of research towards better design, an excellent forum was provided for the presentation and consideration of the Association's particular contribution in this field, which has always been strongly directed towards defining the means of achieving more economical design. This has involved a lengthy and painstaking programme of stress analysis, for example, of the stress distributions set up by typical nozzle attachments, in which the deliberate choice of the examination of large-scale specimen vessels was made and to a large extent made possible by the co-operation of manufacturers of pressure vessels. If, to-day, the experimental approach in this field can be more elegant, it would be difficult to say that this possibility does not arise from the knowledge and confidence won from the large-scale testing work. The answers from such work appear, in any event, to have arrived at a time when, to judge from the mood at the symposium of the Institution of Mechanical Engineers, arguments for advancing code-of-practice design methods in the United Kingdom are likely to be sustained, particularly since much has been done by the Association to translate its accumulated research results into a simple design process.

Equipment for the static and pulsating pressure-testing of large vessels has been moved into the new laboratory, where work will continue on the current phase of the programme, in which attention is being given to some further aspects of the concentration of nozzle-stress factors and also to the important problem of the comparative merits of low-alloy steels in pressure-vessel service. A development foreshadowed in the exhibits displayed by this section on the Association's open day is the study of the behaviour under repeated pressure of vessels at high temperature.

The adjacent section of the new laboratory is concerned with the brittle fracture of welded steel structures and accommodates testing rigs for carrying out the Wells's wide-plate test. Basically, this is a tensile test, giving a stress–strain record to fracture, but the rig is designed specifically to accommodate wide (typically 36 in.), thick plates with the facility for cooling to sub-zero temperatures. The specimen plates contain a longitudinal butt weld, and an artificial notch is placed in the zone of the weld, where it is subjected to the heating cycle and the high, local, residual stresses induced by welding. As a means of studying the influence of important technological factors, for example, quality of steel, type of electrode, stress-relieving treatment, etc., the test has already given results of considerable significance to present practice in heavy engineering in which the brittle fracture problem can in certain circumstances be critical. This work has been done with rigs of 700 and 2,000 tons capacity, counterparts of which now exist in other countries, and the satisfactory performance of these has permitted the Association to advance the design to a new rig, exhibited in model form, that will be used for the study of low-alloy steels and which will have a capacity of 4,000 tons tensile load.

Fundamental aspects of brittle fracture in steel, with particular reference to fracture initiation and propagation, are also being investigated. The determination of a generalized resistance of the material embodied in the measurable quantity frac-

ture toughness, has proved a difficult experimental problem in relation to propagation of fracture. Recent work on measurements of transient force against time in the Charpy (impact) test has indicated, however, that this test might well be capable of modification for the particular purpose. The examination of fracture surfaces for the detection of slip patterns, using suitable materials and etching and microscope techniques, has proved extremely fruitful in the study of fracture initiation. By causing the arrest of fracture in a Charpy test at an extremely early stage, a trigger mechanism has been revealed that appears in accord with Cottrell's hypothesis (*Trans. Amer. Inst. Met. Eng.*, **212**, 192; 1958) of a crack resulting from the intersection of two mutually perpendicular slip bands (see *Nature*, **190**, 432; 1961).

The third research section of the new laboratory of the Association is devoted to work on the resistance group of welding processes, namely, spot, projection, seam welding, etc. These methods are normally used in the quantity production of sheet metal components, in which field the problem of maintaining consistency of quality under rapid fabrication conditions often demands a critical examination of material and process control. The section is equipped with a range of modern welding machines and with ancilliary apparatus for the measurement of the important process variables. In addition, techniques have been developed for the direct measurement of temperatures during spot welding. The problem is complicated by reason of the fact that the generation of heat in the production of a spot weld takes place at the interface region of two overlapping sheets, and temperature gradients are severe. A considerable amount of work has been done by the Association to overcome the difficulty by using buried thermocouples, and the results will be of importance in extending the welding process to harder steels, for which a knowledge of heating and cooling conditions is of particular importance.

The investigations mentioned and those being conducted in other departments are described in the sixteenth annual report of the British Welding Research Association, which has just been issued. For the year under review, the Association's income was £265,379, an increase of £53,252 over the year 1959–60. This increase is, in itself, greater than the total income of the Association in the year when the research station was inaugurated, and is evidence of the rapid growth of the Association to a position in its field which is probably unequalled outside the U.S.S.R.

R. P. Newman

THE NUFFIELD FOUNDATION

THE sixteenth annual report of the Nuffield Foundation*, covering the year ended March 31, 1961, in which grant allocations exceeded £2 million, comments on the extent to which the Foundation sometimes participates in the management of the programmes it supports, thus keeping closely abreast of present-day thought and discovery in its fields of interest. Sometimes its neutrality and independence enable it to take the initiative in bringing together many varied, and sometimes conflicting, interests. Again, it can also, as in the establishment with the Joseph Rowntree Memorial Fund of a National Institute for Social Work Training, or in planning a medical school for the University College of Rhodesia and Nyasaland, draw on its long and varied experience to render important advisory services. Furthermore, its activating role is illustrated by the promotion, at the invitation of the Colonial Office, of an informal but widely representative study of the problems associated with the provision of an adequate and proper supply of British television material to television stations overseas, particularly to those in the less-developed countries. Two conferences were convened for such a purpose by the Foundation, and the resulting document, summarizing the broad conclusions of the study, and making detailed and costed proposals for action, may result in the establishment of a special organization in Great Britain to promote the supply of British television material to overseas countries by all possible means.

Of the allocation of £165,000 for science, including technology, £50,000 went to the Madingley Field Station for the Study of Animal Behaviour, Cambridge, for new buildings and recurrent expenses up to ten years, and £36,000 over five years to the Department of Medical Biochemistry and Pharmacology, Birmingham, for work under Prof. A. C. Frazer on the biological effects of oxidized fats. An additional grant of £3,500 a year for four years to the Department of Chemistry, King's College, Newcastle upon Tyne, in support of Prof. J. Baddiley's work on the structure of cell walls will enable investigations into the biochemical aspects of the cell wall and teichoic acids to be initiated, and a grant of £11,500 over three years is in support of the microbiological aspects of Prof. C. H. Hassall's work at the University College of Swansea on the biosynthesis of complex phenolic compounds; a grant of £4,950 to the Gatty Marine Laboratory, University of St. Andrews, will provide Dr. A. J. Matty with research assistance over three years in his investigations of the thyroids of lower vertebrates.

In technology, a grant of £3,250 for five years was made to the National Trades Technical Societies of Sheffield to assist the introduction of their organization and educational services for adult workers in industry to other industrial areas; £15,000 to the Loughborough College of Technology will provide four postgraduate studentships in ergonomics for each of the years 1961–64 in the newly created Department of Ergonomics and Cybernetics; £5,500 to the Department of Mathematics, Royal Technical College, Salford, is to finance an investigation of mathematics in the electrical engineering industry; and an additional £1,200 to the Department of Chemistry and Food Technology, Borough Polytechnic, will allow Mr. P. D. S. Wood to continue the work on lipids until Easter 1962.

Of grants totalling £600,000 for medical projects, £220,000 was to meet the capital cost of the Nuffield Institute of Nuclear Medicine and running costs in its early years, while, also at the Middlesex Hospital Medical School, up to £45,000 during its first seven years goes to the establishment of a new research unit

* The Nuffield Foundation. Report for the year ended 31 March, 1961. Pp. xv+210. (London: The Nuffield Foundation, 1961.)

in biology as applied to medicine. £60,000 to the University of Manchester is to provide the Department of Audiology and Education of the Deaf with more accommodation for clinical and research work for the young deaf child, and a further grant of £29,350 over three years is to go to the audiology unit at the Royal Berkshire Hospital for work on hearing and deafness in early infancy. A capital grant of £70,000 has been made for an extension to the Liverpool School of Tropical Medicine to further co-operation with schools of tropical medicine and endemic disease in Ghana and Thailand, and a further £40,000 to the Zoological Society of London for building the Nuffield Institute of Comparative Medicine. A grant of £30,000 was made to the Central Council for Health Education for an investigation into the present patterns of sexual behaviour and attitudes towards sex and marriage among young people between the ages of thirteen and twenty, which is expected to take three years to complete; one of £6,600 over three years to cover the salary of a research biochemist to work under Prof. G. Belyavin at University College Hospital Medical School on the response of selected cell lines to infection with influenza virus; and another of £3,200 a year for two years to the Institute of Orthopædics to enable Dr. C. H. Lack to expand his work on the role of plasmin and plasmin inhibitors in the breakdown of connective tissue matrix, especially that of cartilage.

A grant of £25,000 has been made to the London School of Hygiene and Tropical Medicine, mainly to cover the first year's cost of a training course in industrial hygiene under the direction of Prof. R. S. F. Schilling, head of the School's newly created Department of Occupational Health, and the Foundation has also set aside £250,000 specifically for the further development in Britain of practical schemes of co-operation in industrial health and occupational hygiene. Of this, £15,000 has been allocated to provide the Central Middlesex Industrial Health Service, Ltd., with a permanent clinic, and £20,000 on a tapering basis for the re-organization and reconstitution of the Slough Occupational Hygiene Service, on the understanding that the unit can be made self-supporting within five years.

From the £165,000 for social research and experiment, £12,500 for a period of four years went to the scheme for an experimental family welfare centre at Stevenage New Town to cover the salary of the organizer, rent, maintenance and administrative expenses; £2,000 for a study by Prof. T. Ferguson of Glasgow young people who have been in the statutory care of the local authority and particularly of their assimilation into the community; £20,000 over three years for an inquiry by the Nature Conservancy and the Department of Estate Management, University of Cambridge, into the management of common lands; £9,500 over three years to the Women's University Settlement for developments in the after-care of discharged prisoners; £7,600 to the University of Cambridge for an investigation by Mr. Z. A. Silberston into the economic impact of the patent system in Britain; £5,000 to the Institute of Strategic Studies for the development of the Institute's Library, and £5,000 over five years to the London School of Economics and Political Science to enable Prof. W. A. Robson's group to undertake a more comprehensive study of local government in Greater London.

Among grants totalling £220,000 in education, special attention is directed to two of the smallest: £300 towards the cost of a five-day residential meeting at the Battersea College of Technology, arranged by Dr. L. R. B. Elton, to consider policy and future action in regard to the proposed revision of syllabuses and teaching methods for Ordinary- and Advanced-level physics for the General Certificate of Education; and £1,500 in support of experiments in the teaching of French at East Ham Grammar School for Boys and Talbot County Primary School, Leeds. Grants were made of £100,000 to the Royal National Institute for the Blind for the change-over of the Nuffield Talking-Book Library from records to long-playing tapes; £20,000 to the University College of North Staffordshire for provision of multiple copies of texts and other documentation for the foundation year with an additional £5,000 towards the cost of an assessment of the foundation year; £30,000 over five years to the University of Cambridge for the continuation of its aerial reconnaissance over the British Isles; £6,650 over two years to the Department of Extra-Mural Studies, University of Liverpool, for a Senior Research Fellow to undertake a review of the nature and extent of adult educational provision at the present time; £4,250 for a critical analysis by the Acton Society Trust of management education courses; and a capital grant of £5,000 to Westminster School to form the "Tizard Research Fund" to assist research activities by the science staff of the School. The grant to Prof. P. L. Krohn's gerontological research unit at the University of Birmingham has been renewed a third time at the rate of £7,000 for two years, and an additional £1,800 allocated for Mr. F. Le Gros Clark's studies of women of mature age in paid employment and of occupational opportunities for people in retirement.

Grants totalling £385,000 for the Commonwealth overseas, distributed in forty grants to fifteen different countries, include £85,000 to finance for the next ten years a scheme at the University of Cambridge for a Nuffield Unit of Tropical Animal Ecology; £35,000 over five years to the Plunkett Foundation for Co-operative Studies for training courses aimed at raising agricultural standards in Africa; £10,000 for a survey with the Colonial Office of the incidence of deafness in East and West Africa; £18,000 over six years to the Cotton Research Station, Namulonge, Uganda, for a study of soil fertility in East Africa; £29,000 over five years to the East African Institute of Malaria and Vector-Borne Diseases for study of the effects of malaria on African vital statistics; up to £20,000 for angio-cardiological research equipment for the University College Hospital, Ibadan; a further £16,400 over two years to the University of Edinburgh to continue the study under Dr. K. L. Little of urbanization and the effects of industrialization in Sierra Leone. A further £19,000 was offered to the University of New South Wales in support of the Nuffield research chair of mechanical engineering for an additional five years; £8,000 over three years to the University of Adelaide for Prof. D. Rowley's studies of natural immunity to infectious disease; and £10,000 to the Young Women's Christian Association, Belize, British Honduras, for repayment of a loan for a community centre and hostel for girls.

Allocations to fellowships and scholarship schemes totalled £205,000. United Kingdom awards in medicine included three Heinz fellowships of the British Pædiatric Association, four scholarships at University College, Ibadan, two medical fellowships, and four scholarships and three fellowships in

dentistry. One scholarship and two bursaries in biology were awarded, and four senior awards made in sociology. Under the schemes for the Dominions and Colonies there were eleven awards in medicine, fourteen in natural science (including one in engineering) and seven in the humanities and social sciences, in addition to ten Canadian travel grants and twelve farming scholarships (three for Africans). Two travelling fellowships were given to Indian Civil servants and fifteen Royal Society and Nuffield Foundation Commonwealth bursaries were awarded, while 124 grants were made from the Elizabeth Nuffield Educational Fund covering a wide range of projected careers.

EFFECTS OF IONIZING RADIATION ON ORGANIC AND BIOLOGICAL SOLIDS

THE Société de Chimie Physique and the British Biophysical Society combined during July 3–4, in the Laboratoire de Chimie Physique, 11 rue Pierre Curie, Paris, to hold an informal discussion on the effects of radiation on organic and biological solids.

Opening the meeting on the first day, Prof. R. L. Platzman (Argonne National Laboratory, Illinois) outlined very clearly the present state of the theory of primary processes, and the recent developments due to Prof. U. Fano. In systems containing strongly interacting atoms, interaction may influence the primary energy absorption. Molecular structure, and particularly the presence of π-electrons, may affect the proportion of energy absorbed by different components in the primary process. In simple molecules, for example, methane, there is no evidence for such selective mechanism. More information is needed, in particular about the influence of water molecules, before this theory can be applied to biological materials.

Prof. M. Magat (Laboratoire de Chimie Physique, Paris) reviewed reactions in the solid state, in particular low-temperature radiation-induced changes such as polymerization, radical transfer and aromatic ring-opening, which occur by free-radical mechanisms. Members of the Paris group dealt in detail with these reactions, for example, Dr. E. Migirdycian and Dr. S. Leach showed that benzene, if irradiated in the solid state, de-cyclizes and adds on aliphatic groups derived from the ethanol with which it is mixed in solid solution. Similar ring-opening was reported by Dr. K. Hyashi and Dr. S. Okamura (Osaka Laboratories, Japanese Association for Radiation Research on Polymers, Osaka) in the case of lactones, when it is followed by linear polymerization, and favoured by a well-ordered crystalline state.

A feature of the meeting was the use of electron-spin resonance to identify and measure the free radicals produced by irradiation. Drs. R. Bensasson, C. Chachaty, M. Bodard and R. Marx (Laboratoire de Chimie Physique, Paris) used the technique to establish the free-radical nature of low-temperature radiopolymerization of such monomers as acrylonitrile, and Dr. H. Szwarc (Institut de Chimie Physique, Paris) used it effectively to demonstrate the rapid recombination of radicals which occurs on heating irradiated cyclohexane through its transition point at 186° K. However, other factors may be involved, apart from a phase-change, in facilitating radical reactions in the solid. Dr. E. Collinson and Prof. F. S. Dainton (University of Leeds), while demonstrating that scavenging of radicals is more effective in the solid and with aromatic compounds, conjectured that this could be due to the π-electron configuration of the phenyl groups. They also found that a glassy solid behaves more like a liquid than a crystalline solid. The important distinction between aromatic and aliphatic compounds was further emphasized in work by Dr. R. Mason (Imperial College of Science and Technology, London) on post-irradiation thermo-luminescence, when the intensity of emission was found to be a thousand-fold greater in aromatic than in aliphatic amino-acids.

Prof. J. A. V. Butler (Chester Beatty Research Institute, London) introduced the second session by stressing the need for a re-appraisal of such terms as 'direct' and 'indirect' effect, 'target', etc., and emphasized that in living systems, the balance is dynamic, and that radiation need be damaging at only one of a large number of places.

A question of continuing interest is the role of oxygen in radiation damage, and a number of contributions dealt with this factor. Drs. A. Müller, G. Hotz, and K. G. Zimmer (Institute of Radiobiology, Nuclear Research Centre, Karlsruhe) found a modified electron-spin resonance signal when bacteriophage was pressed into pellets in such a way as to expel oxygen, and Dr. L. Ehrenberg (Institutionen för organisk kemi och biokemi, University of Stockholm) on irradiating *Agrostis stolonifera* seeds could distinguish two types of electron-spin resonance signal, one of which is closely correlated with oxygen, and is decreased by pre-heating, that is, when oxygen is expelled. Dr. E. L. Powers (Argonne National Laboratory, Illinois) described recent work on bacterial spores, which offer a convenient experimental system for radiation work. Part of the damage is toxic only in the presence of oxygen. This can be estimated directly, and it is also possible to observe the effect of heat by using high linear energy transfer radiation, when annealing occurs during irradiation. The heat generated during heavy-ion bombardment may be an important and neglected factor, and in the work of Dr. T. Brustad (Norsk Hydro Institute for Cancer Research, Oslo), to be mentioned later, may in part explain the apparent lack of effect of additives.

The nature of the increased damage brought about by oxygen is much under discussion, and whereas the signals observed by Dr. L. Ehrenberg are not characteristic of peroxy-formation, Prof. G. Stein, (Hebrew University, Jerusalem) found a signal when polymethylmethacrylate was irradiated in the presence of oxygen which appears to be peroxidic. This system, moreover, provides a convenient method of measuring rates of oxygen diffusion. In certain circumstances, the influence of oxygen may be minimized by limited diffusion; for example, A. B. Robins and Prof. Butler (Chester Beatty Research Institute, London) found that a high dose-rate can obscure the oxygen effect completely in the case of trypsin.

The use of electron-spin resonance in free-radical investigations has already been mentioned, and Prof. D. J. E. Ingram (University College, Keele) outlined how this can be used in the study of proteins, where electron trapping may occur. It may be possible to correlate denaturation of a protein with an interference in this trapping process since the electron-spin resonance signal does not disappear on warming in the normal way. However, it must be remembered that when applying electron-spin resonance to biological materials, water, for example, plays a part which is little understood.

Sulphur compounds are of much interest on account of their use as protective agents. Dr. R. Koch (Freiburg) investigated more than a hundred compounds by electron-spin resonance, but no simple correlations between structure and function can be seen at the present time. The radical nature is considered to be localized on the sulphur atom, and Dr. P. Alexander (Chester Beatty Research Institute, London) and Mr. M. G. Ormerod (Royal Military College of Science, Shrivenham) provided evidence that in the case of nucleoprotein protected by cysteamine, such localization can occur, minimizing the damage. There seems little doubt that the radicals produced on irradiation of keratin are cystine-like (Dr. A. Redhardt and Prof. B. Rajewsky, Max Planck Institute for Biophysics, Frankfurt), but unaccountably, cysteine and related compounds do not appear to protect by a factor of more than two in a system such as yeast, when judged by quenching of electron-spin resonance signals (Dr. B. Smaller, Argonne National Laboratory, Illinois).

In general, it appears that dry solids are more sensitive than wet solids or solutions, and Dr. R. A. Braams (Physical Laboratory, University of Utrecht) reported a ratio of three between dry and wet tendon. Dr. Robins and Prof. Butler found solid trypsin to be more sensitive than its solutions, possibly due to the self-annealing processes in the presence of water. The last-mentioned authors also reported that the radiosensitivity of trypsin is modified by the medium from which it is evaporated and a low *p*H leads to a highly sensitive state, possibly by aggregation. It is significant that at high linear energy transfer Brustad obtained target sizes in excess of the accepted molecular weight for some enzymes; this also raises the question of energy transfer in the solid state. The effect of added materials depends on their being intimately mixed with the substrate, and the state of the solid is thus again seen to be important.

Two papers were presented dealing particularly with chemical changes on irradiation. Dr. W. M. Garrison (Crocker Laboratory, Lawrence Radiation Laboratory, Berkeley) found good evidence for the conversion of the —NH—CH— configuration in amino-acids into keto-acids, and as little as 10^{-8} mole can be detected as derivatives. Dr. H. A. Dieu (Laboratoire de Chimie Physique, Université de Liège) pointed out that irradiation of ovalbumin leads to a more ready attack by proteolytic enzymes, and an 'unmasking' of sulphydryl groups with the heaviest doses. Moreover, since the rate of subsequent attack by pepsin depends on the dose, and that by trypsin does not, it is probable that the aromatic amino-acids have been preferentially destroyed. In the myoglobin molecule it appears that possibly the leucine residues are the foci of damage, in particular those in more-exposed parts of the molecule, and Mr. C. C. F. Blake and Dr. D. C. Phillips (Davy Faraday Laboratory, Royal Institution, London) provided evidence for this in which the effect of radiation was studied directly by X-ray crystallography.

In summing up, Dr. L. H. Gray (Radiobiology Unit, Mount Vernon Hospital, London) stressed that physical theories, such as that of Prof. U. Fano, must be cautiously applied to biological systems, bearing in mind their complexity and such unknown factors as water content. It appears that the degree of crystallinity of the solid phase may be of great importance, and electron-spin resonance has helped to emphasize this. Concerning the role of oxygen, the accessibility of this gas may be a factor that has not received enough attention hitherto.

Finally, I was struck by the different interpretations that were placed on electron-spin resonance signals, and feel that this useful technique needs to be used with reserve when drawing conclusions.

A. B. ROBINS

BACTERIA OF THE INTESTINE

THE summer conference of the Society for Applied Bacteriology was held at Wye College, Ashford, Kent, during July 11–13, and included a symposium on "Bacteria of the Intestine".

Dr. H. Williams Smith, in his paper entitled "The bacterial flora of the fæces of animals with particular reference to its development in the young", described experiments on the development of normal flora in healthy animals. Rectal swabs were shaken in phosphate buffer, and after passing the suspension through muslin, colony counts of *Escherichia coli*, staphylococci and *Clostridium welchii* were made.

Calves, lambs, piglets and a baby were examined daily for 2–3 weeks, weekly for three weeks, monthly for nine months and twelve months (baby). One rabbit was examined weekly.

In the calf, *E. coli*, streptococci and *Cl. welchii* predominated on the first day, but bacteroides domination began at two days and these organisms rapidly multiplied up to fourteen days, when lactobacilli followed. Counts slowly dropped to 10^7–10^8/gm. of fæces after thirty weeks.

Similar results were obtained from lambs and piglets, except that streptococci dominated later with *E. coli* in lambs, and the piglet flora was dominated ultimately by lactobacilli. Results for the baby were similar also, with counts of 10^{11} for *E. coli* at first, followed by a bacteroides count of 10^{10}; *Cl. welchii* rose sharply at twenty-five weeks when the child started eating solid food. The counts remained high after a year; this was different from the animals. *Staph. aureus*, 10^3–10^6/gm, was frequently found in the baby but not in the animals. The rabbit showed a constant bacteroides count of 10^9; the lactobacilli and streptococcal counts were 10^4 and 10^5. Gram-negative anaerobic cocci up to 10^8–10^9, and yeasts in older calves, were also found. There was a constant population of *E. coli* but frequent changes in phage types.

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Adult animals, including cats and dogs, showed a high count of *Cl. welchii* which varied in degree according to the diet, being alternately high and low when fed on meat or bread. Similarly, if pigs were starved and fed alternately the count went down and up accordingly. The floras of adult guinea pigs were dominated by lactobacilli.

Dr. H. Haenel ("Micro-ecological rules in the composition of the intestinal flora of man") said that before birth the baby was germ-free, at birth infection was acquired from the mother's vagina and fæces, and after 3–4 days lactobacilli predominated. The effect of the mother's milk was to replace aerobic lactobacilli by *Lactobacillus bifidus* and to depress clostridia but not *E. coli*. After weaning, *L. bifidus* stayed at a high level, but aerobic lactobacilli and clostridia returned later. Similar results were obtained for poorly developed children in hospital. Factors ruling the flora were easily disturbed; when stools were stored the lactobacilli diminished and streptococci increased and under abnormal conditions there was a reduction of lactobacilli and an increase in proteolytic organisms.

Antibiotics such as terramycin abolished *L. bifidus* and there was an increase in streptococci and aerobic lactobacilli, with an occasional predominance of *Proteus*; the normal flora returned quickly when the antibiotic was stopped. Under antibiotics salmonellæ disappeared but they could return later; they sometimes shared the normal flora without disturbance. After gastrectomy, the anaerobic lactobacilli disappeared, *E. coli* counts became higher than normal; enterococci, clostridia and other organisms increased also and the large intestine showed high counts. In the adult the flora was stable. Normally the small intestine was nearly sterile, and the large intestine showed mostly anaerobic lactobacilli. Under abnormal conditions there could be a predominance of *E. coli* in the small and no anaerobic lactobacilli in the large intestine; the flora might be the same throughout the intestine with anaerobic lactobacilli absent.

Dr. Anne Dickinson and Dr. G. Mocquot ("Studies on the bacterial flora in the alimentary tract of pigs: 1, Enterobacteriaceae and other Gram-negative bacteria"), observed pigs of 20–100 kgm. in individual pens; the diet was supplemented with antibiotics in some animals. The object was to identify Enterobacteriaceae and other Gram-negative flora isolated on deoxycholate citrate agar after 24–28 hr. at 37° C. Details were given of ten groups classified mainly on morphology, colonial appearance, motility, whether mucoid or not, glucose agar stab cultures, lactose fermentation, growth at 45° C., indole, lysine decarboxylase, urea hydrolysis, production of hydrogen sulphide, liquefaction of gelatine, and ornithine and ammonia. There was no change in the distribution of types under antibiotic feeding. Group 1 usually dominated and the other groups varied for no apparent reason.

Drs. P. Raibaud, M. Caulet, J. V. Galpin and G. Mocquot ("2. Streptococci: selective enumeration and differentiation of the dominant groups") described the isolation of 415 strains of group D streptococci from cæcal and fæcal swabs, using their own medium and also those described by Barnes, Slanetz and Kjellander; thirty-eight types could be distinguished by fermentation reactions differing in at least one carbohydrate. Urea hydrolysis was used for differentiation and the results were regarded as of taxonomic significance. The predominant streptococci were related to *Streptococcus bovis* or *Strep. equinus*, but the classification of these organisms is at present extremely confused.

Dr. M. Lev's paper on "Germ-free animals and their use in elucidating the action of the gut flora on the host" was read by Dr. Cuthbert. Using an apparatus consisting of a nylon tube over a frame, all of which could be autoclaved, chickens and turkeys were hatched from washed and sterilized eggs and the young mammals, monkeys and guinea pigs removed by cæsarean section. Sterilized foods with and without antibiotics were given through tubes, and the effect on the germ-free birds and animals noted. Normal chicken fæces fed to germ-free chicks depressed growth, but feeding penicillin depressed *Cl. welchii* and stimulated growth. Feeding *Cl. welchii* to germ-free chicks depressed growth, but the effect was partly reversed by penicillin in the diet; growth was not increased when germ-free animals were given penicillin. Toxins of *Cl. welchii* caused the gut to thicken and this was prevented by penicillin. Feeding lactobacilli, streptococci and *E. coli* to germ-free creatures had no effect, but the addition of *Cl. welchii* depressed growth, which again was partly reversed by penicillin.

Deficiency in vitamin A led to infection, and gross deficiency caused death; lesions could be seen in the germ-free animals. Germ-free animals on a vitamin K-deficient diet suffered prolonged gut bleeding and the blood had no clotting power. Menedian (four vitamins) did not restore the clotting power, and it is suggested, therefore, that gut bacteria are needed to convert the menedian to some form which can be used.

Entamoeba histolytica did not attack the germ-free gut but survived for a few days only when *coli-aerogenes* were present; they did not survive when autoclaved cæcal contents were fed. There appeared to be complex factors involved in harmless organisms promoting a lethal effect in contact with pathogens. *Shigella flexneri* did not attack the normal guinea pig, but killed the germ-free animals; this was prevented by prior feeding with *E. coli*, non-colicine forming and not demonstrably toxic *in vitro*.

Germ-free animals were equally affected by hæmorrhagic shock caused by the endotoxins from gut Gram-negative flora. Germ-free chicks after five weeks exposure to conventional conditions suffered severe setbacks. Tests are needed on synthetic diets.

Dr. Joan Taylor, describing the "Host specificity and enteropathogenicity of *Escherichia coli*", said that the typing of pathogenic *E. coli* was based on '*O*' (somatic), '*K*' (surface) and '*H*' (flagellar) antigens. Infantile diarrhœa was due to types 0111, 055 and 026 mainly, which were found in all parts of the world. 0128 and 0127 could be found in the United Kingdom and United States respectively, but they were now beginning to cross-spread.

Epidemiological correlation confirmed the role of these organisms in infantile diarrhœa. With the three major types, it was possible to follow the rise in numbers until diarrhœa occurred, beginning when there was a pure culture and ending when other organisms were re-established. But there were similar occurrences with no symptoms. Volunteer feeding had confirmed the pathogenicity of 0111 in students, prisoners and a baby, and of 055 more feebly in prisoners.

Calf scours was related to *E. coli*, particularly 078, which was widely distributed, and may become

septicæmic. Swine œdema due to 0138 and 0139 was frequent in the United States, United Kingdom and Canada. Enteritis in lambs due to type 078 occurred in the United Kingdom and Australia; hen septicæmia was caused by type 02.

There was little information about the occurrence of these organisms in healthy animals, but the human types 026, 055, 0111 were found in cats, dogs, and kittens without symptoms. Surveys suggested limited distribution in different species and disease in some only.

Infection experiments were carried out on alternate loops of sterile gut, in anæsthetized rabbits; the rabbits were killed 24 hr. later by injection of air. Inflammatory reactions with exudations occurred in the infected loops, but non-infected loops were sterile and normal. Many strains from human babies, also chicks, water, calf and swine strains, gave no reaction, although some calf-strains were positive. Living cells were necessary for infection.

Dr. J. G. Collee, Miss J. A. Knowlden and Dr. Betty Hobbs, in their paper on "Studies on the growth, sporulation and carriage of *Clostridium welchii* with special reference to food poisoning strains", observed that typical *Cl. welchii* type *A* strains were completely β-hæmolytic, and that strains were called non-hæmolytic if the hæmolysis was not complete. Food poisoning strains were non-hæmolytic and resisted 100° C. for 1 hr. 80/80 samples of fæces yielded *Cl. welchii*. 39/50 (78 per cent) stool samples from students gave non-hæmolytic strains, but spores from three (6 per cent) strains only were remarkably heat-resistant, as demonstrated by tests on fæces and tests on sporing cultures. Previous reports had given 2 per cent as the average carrier-rate of non-hæmolytic heat-resistant types in normal people; hospital personnel had shown 20 per cent or more. Heat-resistance tests for spores in stock strains revealed the protective effect of cooked meat, which was most apparent with heat-resistant strains. Preliminary findings indicated that the heat-resistant property of typical food-poisoning strains was genetically determined and was not primarily dependent on the presence of large numbers of spores.

Food-poisoning strains seldom produced spores in cooked foods and it was suggested that food should be streaked on neomycin blood agar for non-hæmolytic anaerobic colonies, which should be picked off, grown in Ellner's sporulation medium and the heat-resistance tested. It should be noted that hæmolysis on blood agar was not entirely consistent and spore yields from Ellner's medium were not reliable. Confirmation was given by slide agglutination with the thirteen antisera available; serological cross-reactions and untypable strains still occurred occasionally. It was possible to type 75 per cent of 298 strains submitted during 1960-1961.

An unusual growth pattern at 50° C., the Phœnix phenomenon, was observed with both heat-sensitive and heat-resistant strains; there was no difference between the growth curves of food-poisoning and non-food-poisoning hæmolytic strains over the normal range of temperature, and both could grow under nearly similar conditions, which accounts for occasional outbreaks caused by the heat-sensitive β-hæmolytic types. These strains were unlikely to survive cooking, but may be protected mechanically by lack of heat penetration or may be introduced and find a favourable anaerobic environment during the cooling period.

It was stressed that the relationship of these observations to the bulk cooking of meat and its known association with *Cl. welchii* poisoning of food merited further investigation.

Dr. Betty Hobbs, describing "The public health significance of *Salmonella* carriers in livestock and birds", considered the carriage of salmonellæ by animals and birds in relation to the contamination of foods provided for human consumption; human carriers now appeared to be less important than animal carriers. Particular attention was directed to poultry and egg products and to the contamination of boneless and carcass meats, horse meat and pet food. A parallel was shown between *Salmonellae* isolated from abattoirs and in local populations.

It was suggested that improved conditions and speed of transport for animals passing from farms to abattoirs and more care and attention over hygiene in processing may reduce the spread of infection among animals and the spread of contamination on carcass meat; also, that the pasteurization of egg products and pelleting of animal foodstuffs, both of which eliminated *Salmonellae*, would help markedly to reduce the introduction of *Salmonellae* into bakeries and farms. The increase in salmonellosis due to exogenous types was most probably due to the introduction of egg products, meat, and now coconut contaminated with these types.

Drs. R. Buttiaux and D. A. A. Mossel ("The significance of organisms of supposed fæcal origin in food and water") suggested that the enumeration of organisms indicative of fæcal contamination was necessary because: (*a*) direct tests for pathogens were difficult and statistically uncertain, and (*b*) repeated absence of indicators gave virtual certainty of absence of fæcal contamination, and also (*c*) guaranteed absence of non-pathogenic fæcal contamination. Human indicator organisms had been preferred hitherto but animal indicators might be equally or more important.

The test should be quantitative and foods classified as stable, that is, dried, or perishable, raw or heated. The indicator organisms should be frequent in the gut and scarce outside, but they must be able to survive outside the enteric environment.

E. coli I was common in fæces but not outside. *E. coli II* was fairly common in fæces but occurred elsewhere also. *Klebsiella*, non-motile and urease-positive, were frequent in human stools, also in swine stools but seldom elsewhere. Lancefield Group *D* streptococci were common in animals but little was known about their survival in the environment. *L. bifidus* and bacteroides were unsuitable.

Dr. J. H. McCoy, introducing the discussion, emphasized the fact that examination of water was chiefly concerned with measures against typhoid fever, dysentery and cholera and none of the bacteria responsible was carried by animals; thus interest was centred on human contamination. The survival of *E. coli* in water meant recent pollution; if these organisms were absent there had been time for pathogens to die, as the survival-rate of *E. coli* was similar to that of pathogens. The treatment of sewage aimed to reduce nuisance, not bacteria; *Salmonellae* could be isolated easily from treated sewage. In food the organisms had opportunity for growth, hence numbers of fæcal organisms at time of delivery bore no relation to the degree of original contamination. In untreated food the presence of intestinal organisms led to an assessment of cleanliness and not to safety; therefore there should be direct examination for pathogens also.

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Dr. N. P. Burman, who continued the discussion, showed tables which gave the optimum growth temperature characteristics of coli-aerogenes from various sources and also the coli-aerogenes and streptococcal flora of shallow wells and mains. IMVIC types other than *E. coli* could be found in a variety of places, for example Wilson's irregular type 6.

Coliforms were stimulated by copper treatment, and *Cl. welchii* could be found in chlorinated water supplies. Streptococci found in river waters were mostly animal types, although many strains on membrane filters at 37° C. were untypable; these were eliminated if incubation at 37° C. was exchanged for 45° C. after 4 hr.

In more general discussion, Dr. Blanche Koelensmid and others expressed satisfaction at the authoritative indication that fæcal bacteria in foods cannot be regarded with the same significance as in water.

VITAMIN B_{12} AND INTRINSIC FACTOR

ABOUT 150 leading workers from forty-five laboratories in fifteen different countries attended the second European Symposium on "Vitamin B_{12} and Intrinsic Factor", organized by Prof. J. Kühnau and Drs. W. Friedrich and H. C. Heinrich, and held in the Institute of Physiological Chemistry in the University of Hamburg during August 2–5. Prof. Kühnau, in his opening address, emphasized the importance of the newly revealed structure of vitamin B_{12} coenzyme, endowing it with remarkable activity. In the first symposium at the same place five years ago a notable feature was the disclosure of the structure of vitamin B_{12}, the culmination of several years work by Dr. Dorothy Hodgkin *et al.* at Oxford. This was then the most complex molecule of which the structure had so far been elucidated. In this second symposium, the climax of years of patient work by Drs. Hodgkin and P. G. Lenhert came just in time for the disclosure of the structure of a still more complex molecule, that of a vitamin B_{12} coenzyme, the form in which the vitamin appears mainly to exist in the animal and human body.

Crystals of the 5 : 6-dimethylbenziminazole cobamide coenzyme were grown and photographed with copper X-radiation while in contact with their mother liquor. A complete X-ray analysis of the structure revealed some interesting differences from the structure of cyanocobalamin, which at present is the official form of the vitamin. The new unsaturated pentose previously reported to be liberated from the coenzyme by mild acid hydrolysis has now been found to be an artefact produced during the hydrolysis from ribose through which adenine is linked to the cobalt in the molecule. There may be a free valency in the coenzyme molecule that could play a vital part in metabolic reactions yet to be elucidated.

These differences in chemical composition and molecular structure between vitamin B_{12} and its coenzyme are reflected in differences in their absorption spectra. H. A. Barker *et al.*, at the University of California, where pioneer work on the coenzyme was done, found that in place of the characteristic sharp band of cyanocobalamin at 361 mμ the coenzyme shows a broader band at about 380 mμ and a very slight band at 340–345 mμ. The fairly broad cyanocobalamin band with maxima at about 520 and 550 mμ is replaced by a very broad B_{12} coenzyme band with maximum at about 520 mμ and a slight shoulder at about 495 mμ. The cyanocobalamin band at about 278 mμ is replaced by an inflexion at 288 mμ in alkaline solution shifting to 284 mμ in acid solution and considered specific for the coenzyme. Characteristic differences in absorption spectra of purine- and benzimidazole-containing coenzyme analogues appear to depend on differences in the strength of the bond between the base and cobalt. There is evidently scope for further spectroscopic studies, including a search for isosbestic points that are so prominent in mixtures of cyano- and hydroxocobalamin, now considered to be artefacts obtained from B_{12} coenzyme in the liver and other body stores. E. E. Gabbe and H. C. Heinrich (Institute of Physiological Chemistry, University of Hamburg) emphasized the importance of the freedom of B_{12} coenzyme from cyanide, which they investigated in experiments using radioactive preparations.

B_{12} coenzymes catalyse various reactions that can be used for their enzymic assays. These include conversion of glutamate to β-methylaspartate by the bacterial glutamate isomerase system, the methylmalonyl isomerase reaction and the conversion of certain glycols to deoxyaldehydes. Experiments with deuterium oxide exclude reaction mechanisms involving the intermediate formation of a free proton. These reactions have been studied in bacterial extracts but the methyl-malonyl isomerase reaction occurs in higher organisms.

These findings apply to different B_{12} coenzymes, of which several have been isolated by H. A. Barker *et al.* They appear to be the most abundant corrinoid compounds in various bacteria (for example, *Propionibacterium*) and in mammalian, including human, livers. However, D. Perlman and his colleagues at the Squibb Institute for Medical Research, New Jersey, obtained only 5,6-dimethylbenzimidazolyl cobamide coenzyme from 30 cultures of *Streptomyces*.

B_{12} coenzymes are highly sensitive to light and cyanide. Photolysis yields hydroxocobalamin, which the minute traces of cyanide always present in blood can partly convert to cyanocobalamin. Polarographic studies of these reactions have been carried out by K. Bernhauer *et al.* at Stuttgart. While body tissues usually contain a mixture of hydroxo- and cyanocobalamins, the latter, being at present the officially accepted form of vitamin B_{12}, has been chosen as the standard of comparison with B_{12} coenzyme by a number of investigators. M. E. Coates *et al.* (National Institute for Research in Dairying, Shinfield) detected no difference between the absorption of B_{12} coenzyme and that of cyanocobalamin (both labelled with cobalt-58) through rat intestine in presence of rat stomach extract. Chick assays showed the coenzyme to have only 78 per cent of the activity of cyanocobalamin when administered intramuscularly, but the coenzyme appeared more effective than cyanocobalamin in restoring hatchability in depleted chick embryos. On the whole the coenzyme does not seem to have any therapeutic advantage over cyanocobalamin.

A. W. Johnson (University of Nottingham), described synthetic approaches to the corrin ring

system by Sir Alexander Todd *et al.* (Cambridge). Using radioactive delta-amino-lævulinic acid, porphobilinogen has been shown to be a common biogenetic precursor of vitamin B_{12} and of the natural porphyrins. Biosynthesis by different pathways has been studied by K. Bernhauer *et al.* (Stuttgart) and by A. Di Marco *et al.* (Laboratori Richerche Farmitalia, Milan).

A valuable indication of an important biological function of vitamin B_{12} was obtained by L. A. Manson (Wistar Institute, Philadelphia), who observed deoxyribose synthesis by vitamin B_{12} in a murine leukæmia cell of thymic origin which by leading to nucleoprotein synthesis may explain one of the roles of the vitamin in cellular metabolism. B. Connor Johnson *et al.* (University of Illinois), using the DR-aldolase system, found no relationship between B_{12} deficiency and deoxyribose biosynthesis by $C_2 + C_3$ condensation, and when investigating the role of B_{12} in thymidine synthesis showed that carbon-14 in glutamate was incorporated into the DNA-thymine of regenerating liver. H. R. V. Arnstein and A. M. White found cyanocobalamin to increase biosynthesis of phenylalanine, tyrosine and valine and concluded that neither general inability to oxidize and reduce one-carbon units nor to synthesize thymine can represent the main metabolic lesions in vitamin B_{12} deficiency.

In the search for specific effects of vitamin B_{12} deficiency much attention has been directed to the production of antimetabolites to the vitamin which could be used to produce and investigate controlled deficiencies. E. Lester Smith (Glaxo Laboratories, Ltd., Greenford) found the amides of the monocarboxylic acid, preferentially produced on mild hydrolysis of B_{12}, to prevent conversion of the vitamin to its functional form, but while they competed effectively with B_{12} for uptake by intrinsic factor and inhibited enzyme systems *in vitro* they produced no changes in the blood or bone marrow. W. Friedrich, H. C. Heinrich and P. Riedel (Hamburg) succeeded in producing still more effective antimetabolites from factor V_{1a} isolated from sewage sludge. The resulting alkanolamine B_{12} analogues showed very high inhibitory activity on the growth of *E. coli* and *Ochromonas malhamensis*. H. Heinrich related B_{12} specificity to the structure and position of the alkanolamine, nucleotide, and side chains in the corrin ring. Some attention is now being directed to human dietary deficiency of vitamin B_{12} which may occur in vegans (strict vegetarians consuming no animal food) and was first described by F. Wokes, J. Badenoch and H. M. Sinclair to the International Congress of Nutrition in Amsterdam in 1954. F. Wokes (Vegetarian Nutritional Research Centre, Watford, Herts) and A. D. M. Smith (Middlesex Hospital Institute of Clinical Research, London) gave at the Hamburg B_{12} Symposium further details of a B_{12} deficiency characterized by low serum B_{12}-levels and neurological manifestations with lesions in the peripheral and central nervous systems, well marked in some vegans, and of slight occurrence in vegetarians with low intakes of animal protein and vitamin B_{12} from eggs, milk and cheese, as well as in about 1 per cent of the general population. In India, where the Vegetarian Nutritional Research Centre has been asked to help, strict vegetarians consuming scarcely any animal food encounter much more marked B_{12} deficiency than is found in vegetarians consuming dairy produce, while meat-eaters encounter no vitamin B_{12} deficiency. To avoid widespread protein malnutrition in India an extra 2 million tons of protein will be needed annually, which should be fortified with B_{12} to improve its biological value.

Vitamin B_{12} seems to be missing from all staple vegetable proteins. Slight traces in certain leaf proteins (for example, alfalfa, turnip tops) are not significant sources in human diets. However, A. Pfau and G. Kallistratos (Max-Planck Institute for Animal Breeding and Nutrition, Mariensee, near Hanover) found that broad bean (*Vicia faba*) plants can take up vitamin B_{12} from nutrient solutions, about 27 per cent being absorbed in 24 hr. from solutions containing physiological concentrations of B_{12} labelled with cobalt-60. The absorbed B_{12} was found mainly in the roots (87 per cent of total absorbed) and much less in the leaves (3 per cent). In the ensuing discussion F. Wokes reported that in an investigation at Watford, using plants of Russian comfrey (*Symphytum peregrinum*), 200 μgm. per gm. total solids were found in roots and 76 μgm./gm. in total solids in leaves after 24 hr. immersion of the undamaged roots in 50 ml. of aqueous solution containing 40 mgm. B_{12}. These findings may find application in manuring, especially with sewage sludges rich in B_{12}.

The Symposium agreed that the term hydroxocobalamin should be used to describe products in which the cyanide group of cyanocobalamin is replaced either by —OH (in alkaline solution) or by $[H_2O]$, hitherto termed aquocobalamin (in acid solution). Hydroxocobalamin obtained by photolytic decomposition of vitamin B_{12} coenzyme is present in body tissues in amounts at least equal to those of cyanocobalamin. R. F. Schilling *et al.* (University of Wisconsin) found hydroxocobalamin to be absorbed as efficiently as cyanocobalamin and better retained. After intramuscular injection hydroxocobalamin gives much higher serum B_{12}-levels than are given by cyanocobalamin. (G. B. J. Glass and colleagues, New York Medical College.)

Hydroxocobalamin is at present excluded by stringent spectroscopic tests from the cyanocobalamin which is recognized officially as vitamin B_{12} in leading pharmacopœias. Because of the increasing importance attached to hydroxocobalamin its acceptance as an alternative and perhaps also improved form of vitamin B_{12} seems likely to take effect during the near future, partly for the more efficient treatment of pernicious anæmia, but especially to cope with demyelinating diseases or tobacco amblyopia in which cyanide may be an important factor.

Vitamin B_{12} deficiency occurring through lack of intrinsic factor has led to intense efforts to purify and isolate intrinsic factor. L. Ellenbogen (Lederle Laboratories, American Cyanamid Co., Pearl River, New York) reported on the purest preparation so far obtained, active in doses of 50 μgm. or less, with very high binding capacity for B_{12} and B_{12} coenzyme, very low carbohydrate content, no peptic activity and only trace amounts of blood group substances. W. W. Bromer and E. O. Davisson (Lilly Research Laboratories, Indianapolis) obtained intrinsic factor preparations, effective in treatment of pernicious anæmia in daily doses of less than 100 μgm., by *XE*-64, followed by calcium phosphate gel, chromatography, combination with B_{12}, ultra-centrifugation and electrophoresis. R. Gräsbeck and colleagues from the Minerva Institute for Medical Research, Helsinki, used DEAE cellulose column chromatography with gradient elution and found one major B_{12}-binding peak that, under present conditions, is sole carrier of intrinsic

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factor activity. A 'releasing factor' similar to that in the fish tapeworm which liberates B_{12} bound to human gastric juice has been found in human small intestine, kidney, liver, thyroid, heart muscle, brain, spleen and lung, and has been partially purified with the aid of DEAE cellulose chromatography.

Radioactive vitamin B_{12} used in many investigations on its absorption, storage and utilization is available with different labellings. R. Gräsbeck (Helsinki) described how earlier use of cobalt-60 involved large doses of radioactivity. These are considerably reduced when it is replaced by cobalt-58 but solutions of this are unstable. The recently introduced cobalt-57 has several advantages in clinical diagnosis, when either urinary or fæcal excretion, hepatic uptake, plasma radioactivity or total body retention may be measured. Different forms of radioactive B_{12} have also been used to study complex problems concerned with the action of vitamin B_{12} as well as difficult questions regarding the possible role of vitamin B_{12} and its absorption with or without the aid of intrinsic factor.

The last five papers dealt with relationships between vitamin B_{12} and folic acid. J. R. Guest and D. D. Woods (Microbiology Unit, Department of Biochemistry, University of Oxford) described metabolic interrelationships in the synthesis of methionine by *E. coli* and postulated two pathways for the methylation of homocysteine, the first relatively independent of cobalamin metabolism and with a strict requirement for a conjugated folic acid co-factor, the second dependent on cobalamin but functioning with pteroylmonoglutamates as well as conjugate forms. L. Jaenicke (Biochemical Institute, Munich) described how the structure of different forms of folic acid affects their efficiency in the first pathway. D. L. Mollin and A. H. Waters (Postgraduate Medical School, London) found uncomplicated folic acid deficiency completely cured by very small doses of folic acid (50–150 μgm. daily) in persons with megaloblastic anæmia due to idiopathic steatorrhœa who absorb B_{12} normally, obtain adequate amounts of B_{12} in their diets and excrete very large amounts of formimino-glutamic acid in their urine. In patients with idiopathic steatorrhœa and tropical malnutrition syndrome there is defective absorption of both folic acid and vitamin B_{12}. The very low serum B_{12}-levels may be raised (but not to the normal range) by small doses of folic acid which completely remove other effects of deficiency. E. V. Cox *et al.* (General Hospital, Birmingham) found serum iron high in untreated megaloblastic anæmia and reduced to normal after satisfactory therapy with B_{12} or folic acid. Blood 'folic acid' was temporarily increased, especially in patients with adequate iron utilization and status. In some patients with subnormal serum B_{12}-levels, folic acid administration raises these levels; in others it either has no effect or still further reduces them.

Round-table discussions, to which all members listened, were held on the nomenclature of B_{12} vitamins, intrinsic factor assays and standardization of diagnostic radioactive B_{12} absorption tests, vitamin B_{12} pool, turnover-time and requirements, stability of radioactive B_{12} and B_{12} therapy, under the guidance of Dr. H. A. Barker, Prof. K. Bernhauer, Dr. Dorothy Hodgkin, Prof. A. W. Johnson, Dr. S. K. Kon, Dr. Rosenblum, Dr. R. P. Schilling, Dr. Lester Smith and Prof. L. S. Witts. Full details will be published in the Symposium Proceedings.

MEDIATION OF DIVALENT METAL IONS IN THE BINDING OF TETRACYCLINE TO MACROMOLECULES

By Dr. KURT W. KOHN

Clinical Pharmacology and Experimental Therapeutics Service, General Medicine Branch, National Cancer Institute, Bethesda, Maryland

SEVERAL biological and biochemical effects of the tetracycline antibiotics depend on the presence of divalent metal ions. These effects include the inhibition of certain enzymes[1], the precipitation of β-lipoproteins[2], and the localization of tetracyclines in tumour tissues[3,4]. These phenomena probably involve the formation of tetracycline-metal chelates[5]. Calcium chelation may account for the binding of tetracyclines in newly formed bone[6] and for the interference with calcification caused by these antibiotics[7].

The metal ions may act in these systems by simultaneously binding tetracycline and suitable macromolecular sites. In order to test the possibility that such metal-bridge complexes may generally form with macromolecules having binding sites for divalent cations, we studied the interaction of tetracycline with deoxyribonucleic acid (DNA) and with serum albumin in the presence and absence of divalent cations.

Equilibrium Dialysis. Macromolecule solutions (1·0–1·5 ml.) in bags of ¼-in. transparent cellulose tubing were dialysed (40 hr. at 4°) against 15 ml. of buffers containing tetracycline and various concentrations of divalent metal ions. The transparent cellulose bags were weighed before and after dialysis and appropriate corrections were made for any changes in macromolecule concentration. Donnan effects were estimated to be negligible. Tetracycline concentrations inside and outside the dialysis bags were determined by fluorometry of extracted calcium-barbital complexes[8].

The preparations of DNA used were relatively undenatured, as indicated by the absence of any lowering of optical density (260 mμ) in response to 0·01 magnesium chloride[9]. Crystalline human serum albumin was dissolved in 0·15 *M* sodium chloride containing 0·01 *M* ethylenediaminetetraacetic acid and dialysed extensively at 4° against 0·12 *M* sodium chloride plus 0·03 *M* sodium bicarbonate–carbon dioxide buffer at *p*H 7·4. The concentration of the dialysed albumin solution was determined gravimetrically on heated trichloracetic acid precipitates[10]. (Salmon sperm DNA and crystalline human serum albumin were purchased from the Mann Research Laboratories, New York. Calf thymus DNA was obtained from the Worthington Biochemical Corp., Freehold, New Jersey.)

Little or no tetracycline became bound to either DNA or albumin in the absence of divalent metal

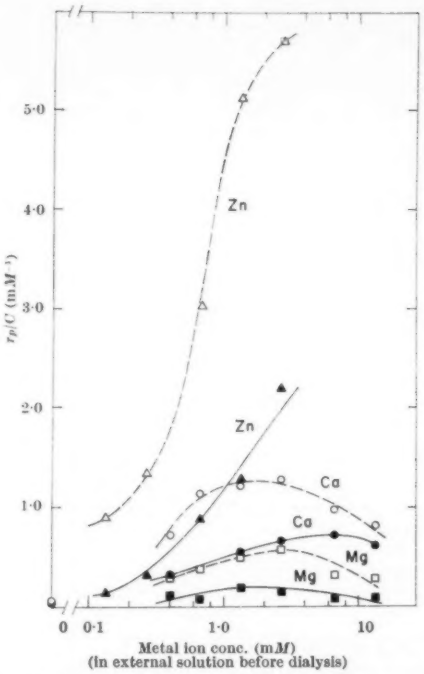

Fig. 1. Effect of divalent metal ions on the extent of tetracycline binding by 'native' (——) or heat-denatured (– – –) DNA, as determined by equilibrium dialysis of salmon sperm DNA solution (2 mgm./ml. in 10^{-3} M potassium chloride) against 10 volumes of 10^{-4} M tetracycline in 0·04 M *tris*-hydrochloric acid buffer at *p*H 7·8 (23°). Ionic strength 0·06 was maintained with potassium chloride. (r_p = mean number of tetracycline molecules bound per DNA phosphorus; C = concentration of unbound tetracycline at equilibrium)

ions. Binding to DNA did take place in the presence of zinc, calcium, manganese or magnesium, in decreasing order of effectiveness (Fig. 1). Heat-denatured DNA (100° for 15 min.) exhibited markedly increased binding. The results were qualitatively similar for DNA derived from salmon sperm or calf thymus. In the case of serum albumin, tetracycline binding occurred in the presence of zinc, but no significant binding was detected with calcium (Fig. 2).

At the low concentrations used, the amount of tetracyline bound was proportional to the concentration unbound. The maximum binding, represented by the highest points on the curves with zinc in Figs. 1 and 2 corresponded to 1 molecule tetracycline bound per 120 nucleotide units of heat-denatured DNA and 1 molecule bound per 40 molecules of albumin, respectively.

Fluorescence. The tetracycline chelates of calcium and zinc fluoresce in aqueous solution. DNA and albumin were observed to enhance this fluorescence. Tetracycline (10^{-5} M) was titrated with zinc chloride or calcium chloride at several *p*H values between 6·5 and 8·0, and fluorescence intensity (excited at 405 mμ, observed at 530 mμ) was plotted against the amount of metal added. The presence of DNA or albumin in such titrations caused much greater fluorescence to appear on addition of the metal (Fig. 3). The macromolecules did not, however, increase fluorescence in the absence of divalent metal ions. DNA and albumin gave qualitatively similar

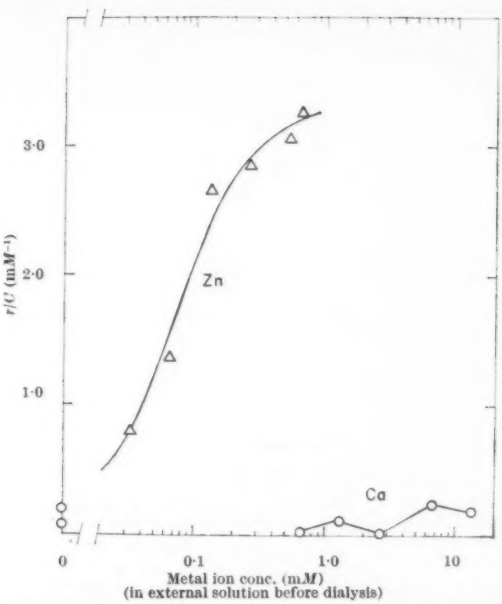

Fig. 2. Binding of tetracycline to human serum albumin. Equilibrium dialysis of 1·0 ml. protein solution (*c.* 30 mgm./ml.) against 15 ml. of 10^{-4} M tetracycline at *p*H 7·52 ± 0·04 (23°) (0·12 M sodium chloride, 0·03 M sodium bicarbonate–carbon dioxide buffer). (r = moles of tetracycline bound per mole of protein; C = concentration of unbound tetracycline at equilibrium)

results. Heat-denatured DNA produced greater enhancement of fluorescence than did the undenatured material.

In other experiments, the concentration of macromolecules was varied while other factors were held constant (Fig. 4). Zinc or calcium were present in sufficient excess to assure nearly complete chelation of unbound tetracycline. Fluorescence enhancement

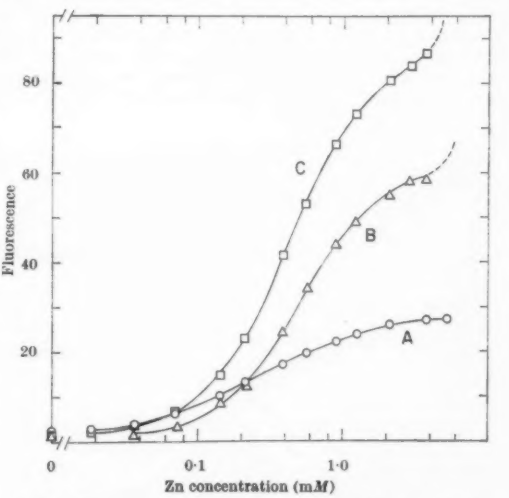

Fig. 3. Fluorescence titration of tetracycline with zinc chloride (0·06 M NaAc–HAc buffer, *p*H 6·65 ± 0·04). *A*, in the absence of DNA; *B*, in the presence of salmon sperm DNA (1·4 mgm./ml.); *C*, same with heat-denatured DNA. (Above 4 mM zinc, the DNA solutions became turbid and the fluorescence rose sharply)

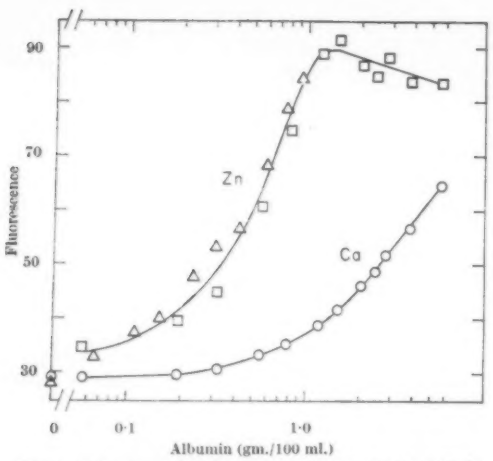

Fig. 4. Enhancement of fluorescence of tetracycline chelates by human serum albumin. Solutions of 10^{-4} *M* tetracycline in 5·8 gm. per cent albumin at pH 7·6 (0·04 *M* sodium bicarbonate–carbon dioxide, 0·12 *M* sodium chloride) and in buffer without albumin were mixed in various proportions. Both solutions also contained either 10^{-3} *M* zinc chloride or 10^{-3} *M* calcium chloride. (Activation at 405 mμ, fluorescence measured at 530 mμ)

occurred at much lower macromolecule concentrations in the presence of zinc than with calcium. The results for DNA and albumin were again qualitatively similar. The rise in fluorescence occurred at lower concentrations with denatured DNA than were required with 'native' DNA.

The enhancement of tetracycline chelate fluorescence produced by DNA and serum albumin might be explained by: (1) binding of tetracycline chelates to specific macromolecular sites; (2) electrostatic attraction of tetracycline chelates into the hydration layers of the macromolecules; or (3) increases in viscosity of the solutions. Electrostatic effects alone cannot account for the large differences in the effectiveness of albumin (or DNA) in enhancing the fluorescence of the zinc versus calcium chelates (Fig. 4), since, under the conditions of these experiments, nearly all the unbound tetracycline was in the form of 1 : 1 chelates which should have the same charge with zinc as with calcium. If viscosity were the major factor, undenatured DNA solutions, which have much higher viscosity than denatured solutions, should produce the greatest increases in fluorescence. The reverse was found to be the case. It appears, therefore, that the changes in fluorescence result mainly from binding which, at least in the case of the zinc complexes, occurs at specific macromolecular sites.

The divalent metal ion probably mediates this binding by serving as a connecting bridge between macromolecule and tetracycline. This conforms with previously reported instances of metal-mediated binding of chelating agents to proteins[11,12].

The results of both the dialysis and fluorescence experiments indicate that the binding of tetracycline chelates to DNA is increased after heat-denaturation. This is in accord with the increase in cation-binding ability brought about by heat-denaturation of DNA[13]. Serum albumin was observed to bind tetracycline in the presence of zinc, but not of calcium. The albumin sites for zinc are probably imidazole groups[14] which would be expected to have very little affinity for calcium. These relationships support the picture of a bond between DNA (or albumin) and the metal component of the tetracycline chelate.

The results strengthen the view that the tetracyclines may exert some of their biological effects by complexing with macromolecules through metal ions. The binding of tetracyclines in human plasma, for example, may be through this mechanism, since purified serum albumin, by itself, does not bind these compounds.

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DAMAGE IN STAINLESS STEEL DUE TO FISSION FRAGMENTS

By P. R. B. HIGGINS and A. C. ROBERTS

Metallurgy Division, Atomic Energy Research Establishment, Harwell, Berks

AN investigation has been made by transmission electron microscopy on the changes in the microstructure of stainless steel bombarded by fission fragments at 60° C. The steel used was a niobium-stabilized 20 per cent chromium, 20 per cent nickel austenitic stainless steel which was received as hot-rolled strip, 0·020 in. thick. The steel had been vacuum-melted twice prior to rolling and its analysis is given in Table 1.

The material was reduced to foil 0·001 in. thick by successive heat treatments and cold rolling. This was followed by a solution heat treatment at 1,145° C. for 20 min. and a water quench. The foils were then thinned electrolytically in a stabilized phosphoric–sulphuric acid polishing solution (solution kindly given by Messrs. Electropol, Ltd., Farnham, Hants), and given an electrolytic cleaning treatment in a mixture of 75 per cent sulphuric acid, 18 per cent glycerol and 7 per cent water. The thickness of the prepared foils was determined by measurements of

Table 1

	Cr	Ni	C	Mn	S	P	Si	Nb	Fe
Chemical composition (per cent)	19·6	20·1	0·02	0·72	0·004	0·008	0·69	0·44	~58·4

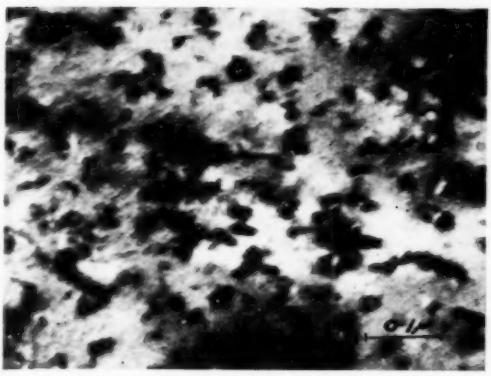

Fig. 1. Damage observed in stainless steel after an irradiation of $8{\cdot}4 \times 10^{15}$ fission fragments cm.$^{-2}$ at a rate of $1{\cdot}6 \times 10^{10}$ fragments cm.$^{-2}$ sec.$^{-1}$

slip traces and found to be a few hundred angstrom units.

Individual foils were held between strips 0·001 in. thick of either natural uranium or 93 per cent uranium-235 by an aluminium clamp. This assembly was sealed in silica and irradiated in the *Bepo* reactor in a thermal neutron flux of $1{\cdot}3 \times 10^{12}$ n. cm.$^{-2}$ sec.$^{-1}$. After irradiation it was necessary to clean the steel surface in the cleaning bath already described; however, this treatment did not remove a significant fraction of the steel from the surface. The steel was then examined by transmission microscopy in an Elmiskop I operating at 100 kV.

The fission-rate (R) in uranium of unit area and thickness y is given by $R = \varphi\sigma y n$, where φ is the thermal neutron flux, σ is the capture cross-section of uranium-235 (590 barns), and n is the number of uranium-235 atoms per unit volume.

Since the distance travelled by a fission fragment in uranium is about 4×10^{-4} cm., it is only necessary, in the arrangement used, to consider the fissions occurring in a layer of this thickness next to the steel surface, and geometrical considerations show that fragments from approximately 30 per cent of these fissions will pass through the foil. Each fission results in two fragments, and since there is uranium on both sides of the steel the flux of fission fragments is given by: $\varphi_f = 4{\cdot}8 \times 10^4\ \varphi\sigma n$ cm.$^{-2}$ sec.$^{-1}$.

For natural uranium this gives a flux of $1{\cdot}2 \times 10^8$ fragments cm.$^{-2}$ sec.$^{-1}$ and for 93 per cent uranium-235 a flux of $1{\cdot}6 \times 10^{10}$ fragments cm.$^{-2}$ sec.$^{-1}$.

Small defects, about 25 Å. in diameter, are first seen in the steel after a fission fragment dose of $1{\cdot}8 \times 10^{14}$ fragments cm.$^{-2}$. Increasing the dose results in an increase in size and number of defects until they are resolvable as dislocation loops, similar to those observed in other face-centred cubic metals after neutron irradiation or quenching from a temperature near the melting point. Fig. 1 shows a typical damaged area after a dose of $8{\cdot}4 \times 10^{15}$ fragments cm.$^{-2}$ at the higher dose-rate.

The steel will also be subjected to neutron irradiation, and to determine how much of the observed damage was due to neutron irradiation similar foils were irradiated at 60° C. in a thermal neutron flux of $1{\cdot}3 \times 10^{12}$ neutrons cm.$^{-2}$ sec.$^{-1}$ (epithermal flux 3×10^{11} neutrons cm.$^{-2}$ sec.$^{-1}$) for 700 and 2,000 hr. No defects were observed in the electron microscope, but tensile tests show a characteristic increase in yield stress.

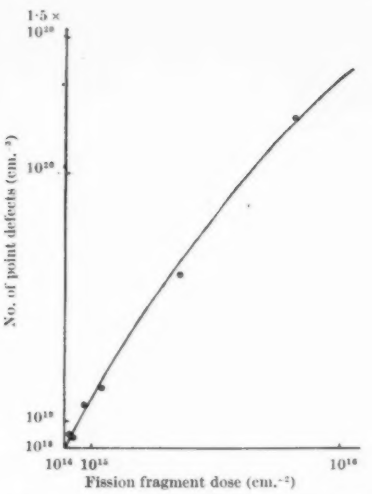

Fig. 2. Increase in concentration of point defects with dose of fission fragments

An estimate of the number of defects observed in the foil has been made by measurement of the integrated area of the defects and a knowledge of the thickness of the foil. Fig. 2 is a plot of the concentration of point defects against fission-fragment dose at a dose-rate of $1{\cdot}6 \times 10^{10}$ fragments cm.$^{-2}$ sec.$^{-1}$. It will be seen that saturation has not been reached at a concentration of $1{\cdot}2 \times 10^{20}$ point defects cm.$^{-3}$. A possible reason for such a large number of defects without saturation has been put forward by Barnes (unpublished work), who suggests that the observed defects are due to interstitial atoms[1]. However, after a dose of $1{\cdot}7 \times 10^{16}$ fragments cm.$^{-2}$, the dislocation loops appear to have grown to form the dislocation network shown in Fig. 3, which may mean that saturation has been reached at this dose.

The number of pairs of point defects produced by each fission event has been obtained from the slope of the initial part of the curve of Fig. 2, which gives a maximum of 45. This is 2 or 3 orders of magnitude lower than the estimated number of defect pairs

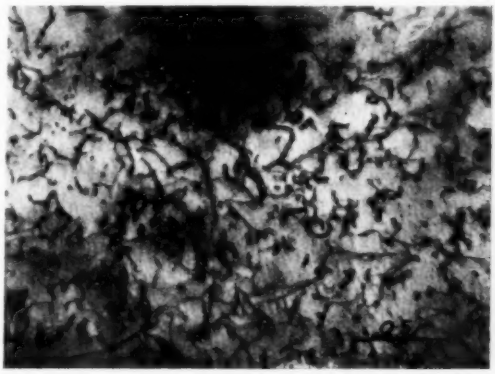

Fig. 3. Dislocation network observed in stainless steel after an irradiation of $1{\cdot}7 \times 10^{16}$ fission fragments cm.$^{-2}$ at a dose-rate of $1{\cdot}6 \times 10^{10}$ fragments cm.$^{-2}$ sec.$^{-1}$

produced by a fission event[2]. A higher number of defect pairs per fission event can be obtained by either irradiating at a higher temperature or at a lower dose-rate. For example, after irradiating the steel at a rate of $1{\cdot}2 \times 10^{8}$ fragments cm.$^{-2}$ sec.$^{-1}$ at 60° C., the number of pairs of defects per fission is 250. This last figure is of the same order as that found in uranium by B. Hudson, K. H. Westmacott and M. J. Makin (unpublished work), where the observed number of point defects will account for the irradiation growth observed experimentally.

The low number of defects per fission event obtained with the specimens subjected to the higher dose-rate at 60° C. is thought to be due to a large number of unobservable defects being present. Higher temperatures, or lower dose-rates, allow these small defects to grow to observable sizes.

Further experiments to study the effects of dose-rate and temperature of irradiation are in progress and will be reported later.

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ELIMINATION OF THE ABSORPTION CORRECTION IN THE VISCOMETRY OF DILUTE SOLUTIONS OF HIGH POLYMERS

By H. van OENE and Prof. L. H. CRAGG

Department of Chemistry, University of Alberta, Edmonton

In a recent paper[1] we directed attention to the fact that values of both intrinsic viscosity $[\eta]$ and the viscosity slope constant k', obtained in the usual way, may be significantly in error because of an apparent reduction in capillary radius. As Öhrn[2] has demonstrated, this apparent reduction in radius may be due to absorption of polymer from solution; it may also be due in part to other effects[1]. Öhrn has described a method of evaluating the 'absorption correction', but it is discouragingly demanding of time and careful experimentation. We have devised a means of calculating intrinsic viscosity which makes an 'absorption correction' unnecessary even when the 'absorption' effect is large.

Öhrn has shown that the absorption effect on the relative flow-time t_r may be expressed thus:

$$t_r = \eta_r(1 + 4a/R) \qquad (1)$$

where η_r is the relative viscosity (the ratio of the viscosities of solution and solvent) and a is the apparent reduction in R, the radius of the capillary.

From this it follows that, in sufficiently dilute solutions:

$$t_{sp}/c = (1 + 4a/R)[\eta] + (k' + 4a/R)[\eta]^2c + 4a/R.\frac{1}{c} \qquad (2)$$

and

$$\frac{\ln t_r}{c} = [\eta] - (\tfrac{1}{2} - k' - 4a/R)[\eta]^2c + 4a/R.\frac{1}{c} \qquad (3)$$

for, in such solutions:

$$\frac{\ln t_r}{c} = \frac{\ln(1 + t_{sp})}{c} = \frac{t_{sp}}{c} - \frac{\frac{1}{2}(t_{sp})^2}{c}$$

Subtracting (3) from (2), we obtain:

$$\frac{t_{sp}}{c} - \frac{\ln t_r}{c} = 4a/R.[\eta] + \tfrac{1}{2}[\eta]^2c$$

The correction term $4a/R$ has disappeared from the slope. Hence a value of $[\eta]$ that is unaffected by 'absorption' can be evaluated from the slope of the plot of:

$$\left(\frac{t_{sp}}{c} - \frac{\ln t_r}{c}\right) \text{ vs. } c$$

We applied this procedure to Öhrn's very precise data[2] which he obtained with a solution of a polystyrene fraction in toluene. His measurements were made in three different viscometers over a very wide range of concentrations, 0·000470–0·196 gm./dl. Values of t_{sp}/c, $(\ln t_r)/c$, and $\frac{t_{sp}}{c} - \frac{\ln t_r}{c}$, calculated from the data for the concentration range 0·00232–0·196 gm./dl. (ref. 2, Table 5), are collected in Table 1. The marked effect of absorption at the low concentrations is clearly evident in the values of $\frac{t_{sp}}{c}$ and $\frac{\ln t_r}{c}$, as is the greater effect in a capillary of smaller

Table 1. Values of t_{sp}/c, $\ln t_r/c$, and $(t_{sp}/c - \ln t_r/c)$, calculated from Öhrn's Polystyrene–Toluene Data (ref. 2, Table 5)

	Viscometer S_1			Viscometer S_2			Viscometer S_3		
c (gm./dl.)	t_{sp}/c	$\ln t_r/c$	$t_{sp}/c - \ln t_r/c$	t_{sp}/c	$\ln t_r/c$	$t_{sp}/c - \ln t_r/c$	t_{sp}/c	$\ln t_r/c$	$t_{sp}/c - \ln t_r/c$
0·196	2·512	2·042	0·469	2·530	2·055	0·475	2·547	2·066	0·481
0·134	2·408	2·087	0·321	2·429	2·103	0·326	2·452	2·120	0·332
0·0865	2·345	2·135	0·210	2·373	2·158	0·215	2·399	2·180	0·219
0·0552	2·335	2·196	0·139	2·374	2·231	0·143	2·416	2·268	0·148
0·0230	2·324	2·263	0·061	2·399	2·335	0·064	2·483	2·415	0·068
0·0145	2·363	2·324	0·039	2·469	2·425	0·044	2·597	2·549	0·048
0·00732	2·458	2·436	0·022	2·650	2·624	0·026	2·877	2·847	0·030
0·00567	2·496	2·478	0·018	2·734	2·712	0·022	3·007	2·982	0·025
0·00232	2·832	2·823	0·009	3·319	3·306	0·013	3·914	3·896	0·018

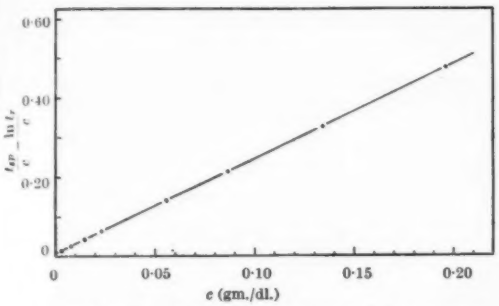

Fig. 1. Plot of $(t_{sp}/c - \ln t_r/c)$ vs. c for data obtained in viscometer S_3. (The S_1 and S_2 data give equally good straight lines parallel to, but slightly displaced from, this line)

radius. A typical plot of $\frac{t_{sp}}{c} - \frac{\ln t_r}{c}$ vs. c is given in Fig. 1.

If the method is valid, it should give the same value of $[\eta]$ for each of the three viscometers even though, because of differing radii, the absorption errors in the three viscometers are very different. In Table 2 are the results obtained, by least-squares analysis, for the data in the concentration-range 0·0230–0·196 gm./dl.

Table 2

Viscometer	Diameters (mm.) $2R$	Slope $\frac{1}{2}[\eta]^2$	$[\eta]$
S_1	0·5	$2{\cdot}34_7$	$2{\cdot}16_9$
S_2	0·3	$2{\cdot}36_9$	$2{\cdot}17_7$
S_3	0·2	$2{\cdot}37_4$	$2{\cdot}18_2$
	Average	$2{\cdot}36_4$	$2{\cdot}17_5$

The agreement among these values of $[\eta]$ is excellent, the maximum difference being 1 per cent (or less than the experimental error in the usual determination of $[\eta]$). Öhrn's corrected values, obtained by evaluation of a and R and application of his correction formula, is 2·14. It differs from our average value and from the value we obtained from the measurements in S_1 (the viscometer in which absorption was least and the capillary radius of which corresponds most closely to that of the viscometers generally used) by less than 2 per cent.

For concentrations less than 0·023 gm./dl., high-precision data are needed, for otherwise the difference between $\frac{t_{sp}}{c}$ and $\frac{\ln t_r}{c}$ would be within the experimental error of either. Since Öhrn's measurements were very carefully made, we calculated values of $[\eta]$ for the concentration ranges 0·196–0·00232 gm./dl. and 0·0230–0·000232 gm./dl., obtaining, respectively, 2·18 and 2·21 dl./gm. Even the latter value is within 3 per cent of Öhrn's value, 2·14.

It is evident that our method makes possible a simple and rapid calculation of a 'true' intrinsic viscosity even when 'absorption' effects are so prominent as to introduce large errors into the values obtained by the usual methods or even to make it impossible to apply the usual methods. It is not necessary either to know the radius of the capillary in the viscometer or to measure (or estimate) the thickness of the absorbed layer. Furthermore, in any dilute-solution-viscosity study in which 'absorption' is suspected, a comparison of the value of $[\eta]$ obtained by this method with that obtained by the usual methods $\left(\text{from values of } \frac{t_{sp}}{c} \text{ or } \frac{\ln t_r}{c}\right)$ will indicate whether or not the 'absorption effect' is significant.

Grateful acknowledgment is made of a grant-in-aid from the National Research Council of Canada and of the award of a fellowship (to H. v. O.) by the Shell Oil Company of Canada, Ltd.

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COLORIMETRIC DETECTION OF PENICILLINS AND CEPHALOSPORINS ON PAPER

By ROBERT THOMAS

Squibb Institute for Medical Research, New Brunswick, New Jersey

A WIDELY used technique for the detection of antibiotics on paper chromatograms and ionograms consists of placing the paper strip in contact with an agar suspension of a sensitive organism and observing the growth inhibition zones which result after a suitable incubation period. Thus, with the aid of the Gram-positive bacterium *Sarcina lutea*, as little as 10^{-2} μgm. benzylpenicillin can be readily detected. However, despite the established value of this bio-autographic procedure for work on penicillin, inherent limitations include the delay necessitated by the incubation period and also the prerequisite of bio-activity, which precludes the possibility of detecting structurally related products which may be biologically inactive. In an attempt to overcome these disadvantages, a colorimetric reagent has been devised which is based on the penicillin iodometric assay procedure of Alicino[1]. This is dependent on the finding that, although the intact penicillin molecule is inert to iodine in neutral aqueous solution, after hydrolysis of the β-lactam ring with alkali or penicillinase the resulting penicilloic acid rapidly consumes nine equivalents of iodine.

This procedure can be easily adapted to paper chromatograms as a two-stage spray consisting of dilute alkali followed by a starch–iodine solution in aqueous acetic acid. Under suitable conditions it was found that both penicillins and the related products cephalosporin *C* and cephalosporin *N* were readily detected as white zones against a dark blue background, with a sensitivity of 1–2 μgm. Thus, chromatogram *A* (Fig. 1) received a preliminary spraying with 0·5 *N* sodium hydroxide then, after an interval of 10–15 min. to allow partial drying, it was further sprayed with a reagent composed of a mixture of 1 per cent aqueous starch, glacial acetic acid and 0·1 *N* iodine in 4 per cent potassium iodide solution (50 : 3 : 1 v/v). Decolorization of the iodine reagent by the hydrolysed penicillins was fairly rapid, yielding maximum contrast after 5–10 min.

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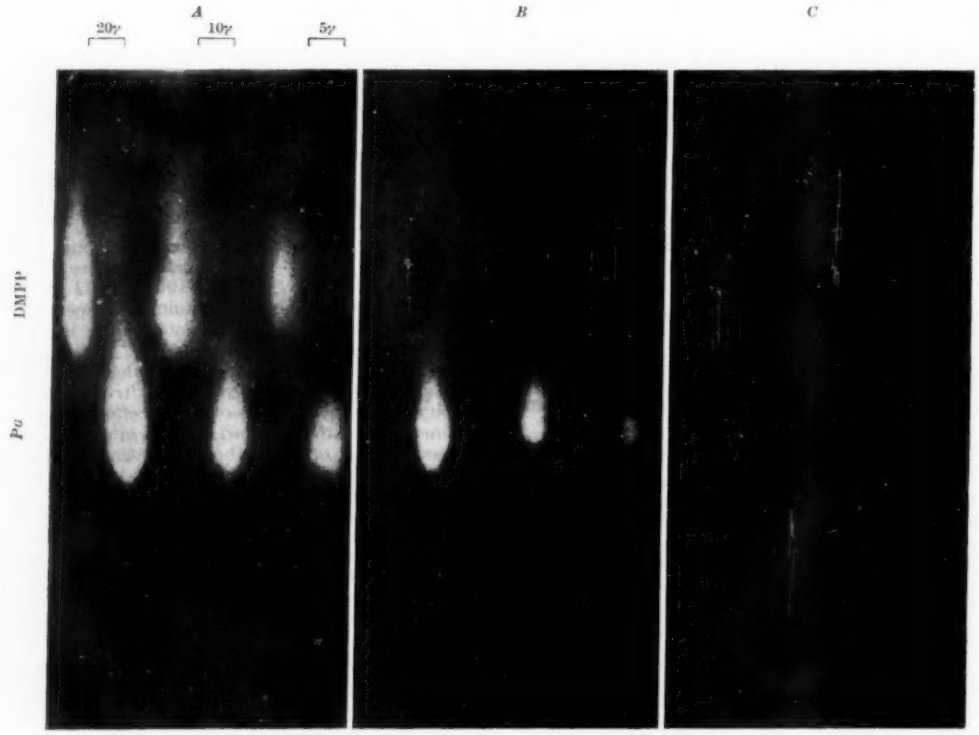

Fig. 1. Chromatograms of benzyl penicillin (*Pc*) and 2 : 6-dimethoxyphenyl penicillin (DMPP) at three concentrations, 20 μgm., 10 μgm. and 5 μgm. Solvent, water-saturated diethyl ether. Whatman No. 1 paper buffered with 10 per cent sodium citrate at *p*H 5·7. After 2 hr. the chromatograms were dried and sprayed with: *A*, 0·5 *N* sodium hydroxide, followed, after partial drying, by a mixture of 1 per cent starch, glacial acetic acid and 0·1 *N* iodine in 4 per cent potassium iodide (50 : 3 : 1); *B*, SchenLabs *B. cereus* penicillinase (10,000 units/ml.), 1 per cent starch, 0·1 *N* iodine, and *p*H 7 *M* sodium phosphate buffer (5 : 50 : 1 : 1); *C*, as in *B*, minus the penicillinase solution

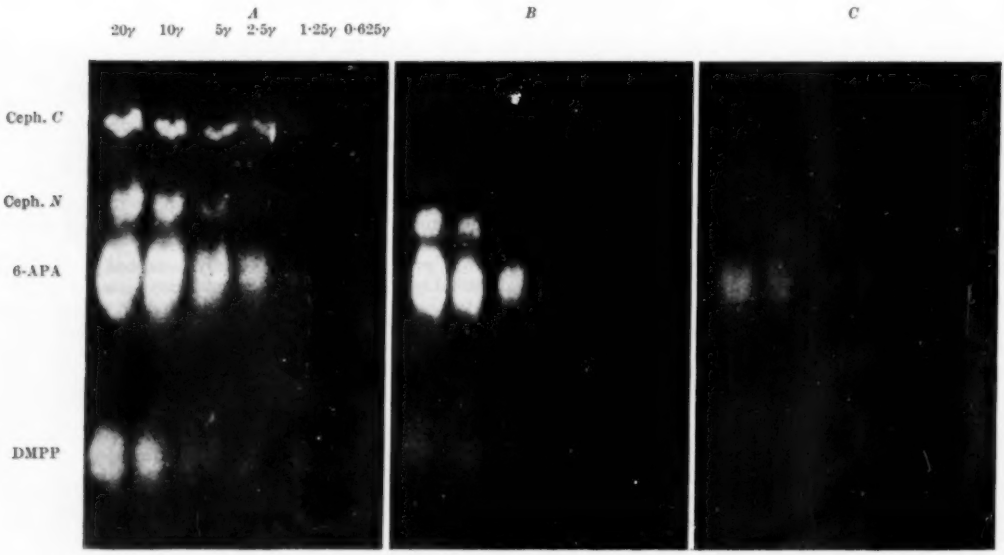

Fig. 2. Chromatograms of 6-aminopenicillanic acid (6-APA), DMPP and cephalosporins *C* and *N* at concentrations between 20 μgm. and 0·625 μgm. Solvent: butanol/acetic acid/water (4 : 1 : 5), run on Whatman No. 4 paper for 4 hr. The three spray reagents *A*, *B* and *C* are detailed in Fig. 1

An identical chromatogram (*B*) was sprayed with an alternative reagent, in which the required preliminary hydrolysis of the β-lactam ring was effected enzymatically with penicillinase. By this means it proved possible to use a single-stage spray for the detection of benzylpenicillin and other penicillinase-sensitive penicillins. The reagent contained a mixture of SchenLabs (SchenLabs Pharmaceuticals Inc., New York) purified *Bacillus cereus* penicillinase (10,000 units per ml.), 1 per cent starch, 0·1 *N* iodine and *p*H 7 *M* sodium phosphate buffer, in the ratio 5 : 50 : 1 : 1. The rate of decolorization of the spray by the penicillin substrate was found to be dependent on the penicillinase concentration and, with this mixture, development was complete at room temperature (c. 22°) in 10–15 min.

It is evident from the chromatogram *B* that, while benzylpenicillin was readily detected by this reagent, 2 : 6-dimethoxyphenylpenicillin was relatively insensitive. This is in keeping with the known partial resistance of this recently described semi-synthetic penicillin (trade names, 'Celbenin' 'Dimocillin' and 'Staphcillin') to the action of the penicillinases of *B. cereus* and *Staphylococcus pyogenes*[2]. The necessity for cleavage of the β-lactam ring is apparent from the negative results obtained when the penicillinase component of the second spray reagent was omitted (see chromatogram *C*).

Thus, in addition to providing a sensitive system for the colorimetric detection of penicillins, by the combined use of the alkali and penicillinase variants of the starch–iodine reagent, it appeared to be applicable to the differentiation of penicillinase-sensitive and -resistant penicillins. This aspect was further examined by a comparison of cephalosporin *C*, known to contain a penicillinase-resistant β-lactam moiety, with cephalosporin *N* and 6-aminopenicillanic acid, both of which are penicillinase sensitive. The results (Fig. 2) clearly show that cephalosporin *C* is even more resistant to penicillinase than 2 : 6-dimethoxyphenylpenicillin.

It can be seen from the third chromatogram (*C*) which, as before, was sprayed with a neutral buffered starch–iodine solution, that in contrast to the results shown in Fig. 1, the penicillinase-sensitive penicillins were still detectable, although with reduced sensitivity. However, since this second chromatographic solvent contained acetic acid then some decomposition of acid-labile penicillins would be anticipated, particularly during the final drying stage, and this could possibly account for the enhanced iodine absorption.

The blue background of the paper chromatogram, after spraying with the penicillinase reagent, faded far more slowly than that obtained with the alkali spray and in both instances the colour was more stable when stored in the dark. In addition, it was shown that the original dark blue colour of the freshly sprayed sheet could be regenerated, even after several days, by spraying again with water.

The sensitivity of the iodometric spray reagent towards certain clinically valuable penicillins is considerably less than that of the bio-autographic procedure. However, for cephalosporin *N*, their relative sensitivities are of the same order and for the detection of both 6-aminopenicillanic acid and cephalosporin *C*, the iodometric reagent is considerably superior when compared with bio-activity against either *Sarcina lutea* or the Gram-negative *Salmonella gallinarum*.

In practice, the sensitivity of these reagents has proved adequate for routine use in the direct examination of chromatograms of mould culture filtrates containing penicillins and cephalosporins. Furthermore, it was found that amino-acids and the normal constituents of corn steep media do not interfere with the detection of these antibiotics in culture broths.

Preliminary experiments have shown that a simple modification of this technique also permits the detection of penicillinase on paper by taking advantage of the ability of the enzyme to render penicillin iodine-sensitive. Thus on spraying initially with a solution of benzyl penicillin (200 units/ml.) followed, after drying, by the *p*H 7 buffered starch–iodine reagent (*C*) described above, *Bacillus cereus* penicillinase could be detected on paper as a white zone against a blue background with a sensitivity of 5–10 ShenLabs units.

An alternative colorimetric reagent for the detection of penicillins has been previously described, which is based on the well-known hydroxamic acid reaction[3]. However, this is much less sensitive than the starch–iodine reagent, which has the additional advantage of permitting the rapid differentiation of penicillinase-sensitive and -resistant penicillins.

I am indebted to Miss Evelyn Albu for her technical assistance.

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ESTIMATION OF SPECIFIC PROTEINS IN SERUM AND OTHER MIXTURES

By Dr. D. A. DARCY

Chester Beatty Research Institute, Institute of Cancer Research: Royal Cancer Hospital, Fulham Road, London, S.W.3

THE purpose of this article is to report the increase in accuracy and simplicity brought about by certain modifications of a method previously described[1]. The method is based on the agar-gel immune precipitation method of Ouchterlony[2]. It consists essentially of placing the protein solution in a hole made in an agar-gel and allowing it to diffuse towards a specific antibody for the protein diffusing towards it from a nearby hole. After the two reactants meet, a band of precipitate is formed composed of both substances. The more concentrated the protein, the faster will the band migrate towards the antibody source, and this phenomenon is used to estimate the concentration of the protein. This is achieved by standardizing the conditions of the reaction and by calibrating the antiserum against a solution of the protein (antigen) which has been selected as the standard.

The details of preparation of the agar plates, the antiserum, the calibration curve, and the incubation

conditions, etc., have been described in the earlier paper: it was shown there that an average error of about ± 14 per cent (95 per cent confidence limits) was obtained routinely. The modifications introduced here reduce this error to less than ± 5 per cent.

Incubation conditions. It was found after numerous experiments that a major source of the ± 14 per cent error was that the gels were not incubated on the level. When ordinary Petri dishes are stacked they invariably tilt a few degrees from the horizontal and this is sufficient to cause large errors. Furthermore, even the bottom plates of the stacks were found to give irregular readings, and this was ascribed to the uneven thickness of their bottoms, which lead to an uneven depth of agar-gel in a given plate. Both these troubles were remedied by the use of flat Petri dishes. It was also found necessary to check (with a small spirit-level) that the platform of the moist chamber on which the plates were stacked was likewise level when in place in the incubator. If a moist chamber consisting of a desiccator jar with a plate glass platform was used, it was found that wrapping the jar with household aluminium foil speeded up the band migration during the 3 days at 37° C.

Preparation of the agar plates. The agar was made up as described previously. No general advantage was found in using lower concentrations of agar than 1 per cent. Although these gave clearer gels and faster migration of precipitates, accuracy was not appreciably increased thereby. Nor, curiously, was readability appreciably increased on these weaker gels—for the precipitate formed was less dense so that the band-gel contrast was about the same as on the 1 per cent agar.

The chief modification introduced in the plates was the use of round holes (wells) in place of the square ones previously employed. The round wells had the advantage that they could be punched out by a commercially available cutter; furthermore, they could be spaced farther apart than the square wells while giving the same density of precipitate, thereby extending the range of protein concentrations that could be measured. The excellent method of Feinberg[2] was followed in making up the plates. A precision cutter and special flat 2·5-in. Petri dishes were obtained from the Shandon Scientific Co. Each dish, after silicone treatment, was filled to a depth of 3 mm. with 1 per cent agar (9 ml.) and allowed to set for a day or more before the wells were punched. The wells were 1 cm. in diameter and the distance between them and the centre (antiserum) well was also 1 cm. (Fig. 1).

Measurement of the precipitate. This was carried out, very simply, with a segment of 'Perspex' ruler enclosing a centimetre ruled in half-millimetres to which a small 'Perspex' handle was attached. The ruled centimetre was laid on the gel surface and lined up so that its limits coincided with the centimetre distance between the centre well and one of the outer wells along a line between their centres (see Fig. 1). The distance from the edge of the outer well to the sharp leading edge of the precipitate was estimated to the nearest 0·1 mm., using a × 2 magnifier and a suitable illumination source. The method is an improvement on that previously used where the rule was laid across the top of the plate, but it is still subject to some parallax error; it is nevertheless capable of a high degree of accuracy as will be seen later. A second method of measurement used in this Laboratory was more elaborate but completely

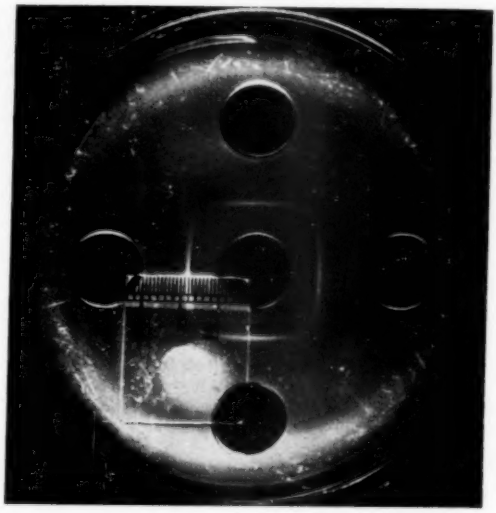

Fig. 1. Standard agar plate showing the centimetre rule lying on the surface of the gel in position for measurement

ruled out parallax. It consisted of using a low-power lens (about × 1) to project the image on an ocular micrometer scale of a centimetre with 0·1 mm.-divisions where it was read by means of an eyepiece (× 7). This gave somewhat greater accuracy than the rule method and has the slight additional advantage of not disturbing the plate.

Experimental test of the method. Accuracy tests were made using rat serum and a rabbit antiserum to the α-globulin of rat serum described earlier[1]. Rat serum yielded with this antiserum one strong band (for the α-globulin) and only traces of other precipitates (Fig. 1). Tests were made with five different concentrations of the rat serum in saline; at the highest concentration the α-globulin band reached nearly to the antiserum well, with the standard conditions, and at the lowest concentration (which was barely in antigen excess) it reached a little beyond midway. Each of the five concentrations was tested against concentrations lower than itself by 5, 6 and 7 per cent. These solutions were presented as unknowns to the person carrying out the test and were indistinguishable by eye. Sixteen separate tests (equivalent to 4 plates) were made on each unknown, using the rule to measure. In every case the method gave a statistically significant discrimination between the solutions differing by 5 per cent ($P = 0{\cdot}01$ or less in every case, except one where P fell just short of 0·01). It was decided to test the method to its limit at the highest of the five concentrations of rat serum, and it was found that a statistically significant discrimination was obtained between the solutions differing by 3 per cent when the rule was used ($P = 0{\cdot}02$–$0{\cdot}01$) and between solutions differing by only 2 per cent when the more elaborate optical method was used ($P = 0{\cdot}05$).

Since, as was seen in the earlier paper[1], accuracy is proportional to the square root of the number of tests made on each unknown, then for the routine method of using only four tests for each unknown the above discriminating power will be halved, or the error doubled. The error for the routine procedure will thus be ± 5 per cent (95 per cent confidence limits)

or less, and at the best ± 2 per cent. For many purposes it may be sufficient to use only a single test per unknown since the error would, on the average, be less than ± 10 per cent. Again with some sacrifice of accuracy, the incubation period could be shortened to 2 days or less if needed.

The method was found to be suitable for other serum proteins such as albumin, for enzymes such as deoxyribonuclease I, but not for xanthine oxidase (mol. wt. 300,000), except possibly if the agar concentration were to be reduced and the incubation time lengthened. The limits of sensitivity are those of the gel-diffusion method, which can at best give a visible precipitate with about 2 μgm. of protein per ml. The method is also applicable to the titration of antisera, for example, by placing a standard antigen in the centre well and measuring to the sharp edge of the precipitate, which will then be on the side facing the peripheral wells.

My thanks are due to Mr. M. S. C. Birbeck of this Institute for advice on optical matters, and to Mr. Peter Harpel of Harvard Medical School for suggesting the effect of tilt.

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CIRCUMSCRIBING IRRADIATION OF SURFACE STRUCTURES OF CYLINDRICAL ANIMAL AND PLANT CELLS WITH POLONIUM-210 α-PARTICLES

By R. M. BERGSTRÖM, R. F. BLÅFIELD and M. W. BRENNER
Institute of Physics, University of Helsinki, and the Institute of Physiology, University of Helsinki

A STUDY of the effects of ionizing radiation on living tissues provides a valuable supplement to investigation of the function of these tissues. The energy of the radiation may be used for complete or partial destruction of the cell material. It can, however, also be used in studying the normal physiological functions of the cells. To this end, irradiation energy can be used as a stimulus energy, which occasions alterations in many physiological processes.

Use of radiation energy for physiological research on cell functions is favoured by the possibility of reaching microscopic structures within the cell, and of partial destruction of fractions of them. As pointed out by Zirkle and Bloom[1], it complements micro-surgery.

This article is concerned with a method which makes use of a cylindrical polonium-210 α-particle source. This renders possible the destruction or the stimulation of surface structures of cylindrical animal or plant cells. The results of the irradiation of single nerve fibres and cells of some algae will be presented elsewhere. This article refers briefly to the ultrastructural changes in the cells of the algae *Nitella flexilis* obtained by this method.

The aim was to produce significant, graded destruction or stimulation of the surface layers of the living nerve and plant cells, with the view of facilitating study of the alterations in the electrical functions of the cells due to the irradiation. Thus, the choice of the ionizing radiation had to fulfil some basic requirements. The ionizing power of the radiation should be as high as possible in order to produce the structural changes. For this reason, heavy-particle radiation was to be preferred. To reduce the effects to a limited surface layer of cells with a small diameter, the depth of penetration of the radiation in organic tissue should be small. For these reasons, and because the aim was to irradiate the membrane of the cylindrical cells from all directions, polonium-210 α-radiation was considered the most satisfactory choice. The reasons for this decision were based on the high ionizing power of the heavy, charged α-particles and the low penetration in organic material.

The half-life of polonium-210 is 138·4 days and the energy of the α-particles emitted 5·298 MeV. The polonium-210 is deposited on the cylindrical inner surface of a silver ring. The nerve or plant cell is held in the centre of the ring, along the axis of the active cylindrical area (Fig. 1). The radius of the active area is 10 mm., and the height, h, is 3 mm. On beginning the irradiation, the ring must be placed rapidly in position around the material to be irradiated. This is made possible by the provision of a slot for the passage of the cell from the outside to the interior of the ring. Thus the whole periphery cannot be used for the active area since it is reduced by an amount, d, of 7 mm. The active area is 167 mm.^{2}.

The source was supplied by the Radiochemical Centre, Amersham, United Kingdom. Its strength, as stated by the manufacturer, was 1·1 mc. ± 20 per cent at the time of delivery, November 3, 1959. The measurements were carried out 11 months later, the activity then being 0·21 mc. The activity was also measured using the nuclear plate technique. The results of this measurement were consistent with the strength stated by the manufacturer. The nuclear plates were kept in a diametrical plane of

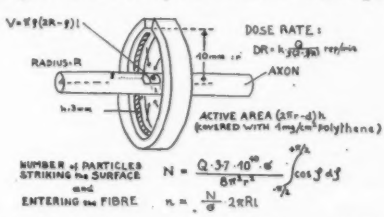

Fig. 1. A schematical representation of the circumscribing irradiation of a nerve fibre from a cylindrical polonium-210 α-source

the active cylindrical surface. By counting the density of the tracks along the cylindrical axis, the intensity of the α-radiation and the activity of the source could be determined.

To avoid contamination, the active area was covered with a 1 mgm./cm.² film of polythene. Strong α-particle bombardment may destroy this and, consequently, it does not provide sure protection. Thus the source was enclosed in a container, from which it could be removed and put in position around the cell by means of a lever. The entire equipment is kept in a special cabinet intended for this particular experiment. Possible contamination originating from the source is detected by means of an end-window type Geiger-counter tube. The window of the tube consists of 2 mgm./cm.² mica.

There are certain advantages in using a cylindrical source. The cell membrane is bombarded equally from all directions. All α-particles pass through equal distances of air and tissue. However, only those particles which enter the surface of the cell perpendicularly will penetrate radially into the cell to a distance which corresponds to the residual range, ρ, in tissue. Evidently, the effect of the radiation is concentrated in only a certain volume, V, of the cell. V is bounded by the surface of the cell and a surface defined by the largest depth of penetration. In order to calculate the dose, we thus have to evaluate the depth of penetration in tissue.

The range in air of the 5·3-MeV. α-particles from polonium-210 is 38 mm. (ref. 2). The loss of energy due to the penetration of the polythene film covering the source is 0·6 MeV. The loss of energy due to the distance covered in air between the source and the cell is 1·1 MeV. Thus the particles entering the tissue have an energy of 3·6 MeV. This value is in accordance with the length of the tracks, observed in nuclear emulsions. The corresponding range in air is 21 mm. (ref. 2) and in tissue 20μ approximately.

Assume that the organic cell is cylindrical and has radius R. Now the volume, V, reached by the particles is:

$$V = [\pi R^2 - \pi(R - \rho)^2]l = \pi\rho(2R - \rho)l \qquad (1)$$

where l is the length of the irradiated part of the cell. Each particle will lose an amount of energy 3·6 MeV. or $5{\cdot}75 \times 10^{-6}$ erg in the cell. If length l is much shorter than the radius, r, of the source, every surface element, σ, of the irradiated part is struck at the same rate of α-particles. We recognize that the height, h, of the cylindrical area is small and thus we can approximate the source by an infinitely thin closed ring. This approximation is regarded as being perfectly satisfactory. The number of particles, N, striking the surface element, σ, in a second can then be written:

$$N = \frac{3{\cdot}7 \times 10^{10}\, Q\sigma}{8\pi^2 r^2} \int_{-\pi/2}^{+\pi/2} \cos\varphi \, d\varphi \qquad (2)$$

φ is the angle between the normal of the surface element and the direction of the particle. Q is the strength of the source given in curies. The rate of α-particles, n, entering the cell is obtained by summing over the surface of the irradiated part of the cell. We get:

$$n = \frac{N}{\sigma} 2\pi R l \qquad (3)$$

The energy dissipation in a unit volume of tissue and in unit time is obtained by combining equations (1), (2) and (3). If we assume that the density of the tissue is 1 gm./cm.³, and divide by 83 ergs, we get the dose-rate in rep/min.:

$$\text{The dose-rate} = 7{,}800\rho \frac{Q}{\left(2 - \frac{\rho}{R}\right)} \text{ rep/min.} \qquad (4)$$

If the nominal strength of the source, 1·1 mc., and the range 20μ are substituted in this equation, we get $4{,}300/(2 - \rho/R)$ rep/min. Because of the inaccuracy of the value for the activity of the source and the error in the residual range, ρ, we expect an error in the dose-rate of ± 15 per cent, as calculated by means of equation (4). It should be pointed out that the calculated dose-rate is a mean value taken over the volume reached by the α-particles. When $\rho < R$, there is a volume in the interior of the cell which is not influenced by the radiation at all. When $\rho > R$, the equation (4) will give too high values, because a considerable number of α-particles escape from the cell.

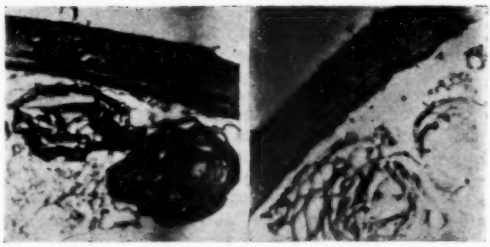

Fig. 2. An electron-micrograph of the cell wall and protoplasm of *Nitella flexilis*, irradiated with polonium-210 α-particles. A normal cell is shown on the left. Magnification: left, × 5,500; right, × 4,400

Here, only one application of the method is described, that is, some ultra-structural changes of the internodal cells of the alga *Nitella flexilis* caused by α-irradiation. The cells, cultured in sea-water from their original environment, were placed in the centre of the polonium-210 ring. Exposure of the cell surface to the α-irradiation was carried out at short intervals, after which the cell was moved to its water environment to prevent it from drying. The whole procedure was performed under a microscope.

In Fig. 2 is shown an electron-micrograph of a *Nitella* cell irradiated with 8,900 rep. The non-irradiated control is shown to the left. The protoplasmic layer of the irradiated cell is separated from the cell wall, whereas it is connected to the wall in the non-irradiated cell. Separation of the protoplasm in the irradiated cell is seen where osmiophilic grains (usually situated near the wall) are displaced to the inner part of the cell. Arrangement of the particles in a row near the wall due to the irradiation with α-particles possibly indicates the existence of a membrane normally connecting the inside of the cell wall. The large chloroplasts, with definite structure in the control, show a diffuse structure with a prominent vacuolization near the cell wall after irradiation.

This work was supported by grants from the Finnish State Committee for Natural Sciences.

We wish to thank Prof. L. Simons for advice and encouragement.

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MUTAGENIC EFFECT OF RADIANT HEAT SHOCKS ON PHANEROGAMOUS PLANTS

By Dr. BROR PETTERSSON
Botanical Museum of the University of Helsinki

In order to prove a possible mutagenic effect of physical and chemical agencies liberated and activated by volcanic eruptions I have made a number of experiments with various phanerogamous plants during the past eight years. The plants chosen for this purpose belonged chiefly to native species, but foreign ones were also represented; these were obtained from the seeds of some Canarian endemics collected by me; there were also fresh herbarium specimens from Madeira, the Cape Verde Islands and Ethiopia. Finally, the material included a few garden plants.

The experiments with the native plants were carried out either on individuals of small island populations easily kept under observation at the place of growth, or on plants of other species settled on an island where they had not grown before. As regards the foreign plants, some were cultivated as common pot-plants and the remainder in garden beds. No particular care was extended to the experimental plants. Pollination control, namely, selfing and crossing, was performed in some cases; otherwise, the rule was free cross-pollination within the population. Only when the fruits were approaching the ripening stage were they protected from scattering by a gauze envelope.

Although the most important occurrence in the evolutionary process of organisms is the genesis of individuals which are distinguished by new characteristics quite different from those characterizing the members of the population within which they had arisen, there can be no question of a new taxon before a mutation has shown itself capable of propagating and stabilizing the new genotype in the environment of the parent population or elsewhere. Accordingly, the aim of my experiments was not only to investigate factors causing mutations in Nature, but also to study the evolutionary pattern of the mutated plants, and the time, measured in generations, necessary for an eventual establishment of new biotypes. Strictly speaking, therefore, the experiments were not exactly genetical in the orthodox sense.

The first stage of this investigation was concerned with the effect of momentary strong radiant heat (such as that caused by incandescent pyroclastic material scattered over surrounding vegetation by an active volcano) on flower buds in various stages of development. In order to obtain the necessary momentary high temperature, use was made of a common burning glass, with a magnification of × 3; by this means, the young flower buds or bud clusters were subjected to the 'burning' rays until some smoke appeared, or until there could be observed a visible change in the colour of the bud, which generally occurred within 2–15 sec.

In conjunction with radiant heat, treatment with sulphur dioxide as sulphurous acid, hydrochloric acid and boric acid was also given in some cases. A small cotton pellet, drenched with one of the solutions, was wrapped round a bud or cluster of buds, kept in this position, and heated during a period of some minutes by means of the burning glass. However, these chemical tests have been too few in number so far to permit any definite conclusion as to differences in mutagenic effect.

The seeds obtained by these rather rough treatments were generally few in number and of inferior germination capacity. The effect of radiant heat could be observed already in the seedlings, principally as teratological deformities. Depending on the severity of the injuries caused by burning, the number of such mutated seedlings varied between 2 and 40 per cent. Frequently, the cotyledons were either already deformed, or their number and position altered. The rather common occurrence of seedlings with three cotyledons was most obvious with *Aichryson dichotomum* (Fig. 1), *Celsia insularis*, *Cynoglossum lanceolatum*, *Dimorphotheca pluvialis*, *Kallanchoë glauca*, *Knautia arvensis*, *Lythrum salicaria* and *Nemophila menziesii*, or with one of them more or less forked; if they were split to the base it was often a difficult matter to distinguish them from true tricotyledons (*Aichryson dichotomum*, *Celsia insularis*, *Centaurea cyanus*, *Kallanchoë glauca*). Of less frequent occurrence were seedlings with only one cotyledon, a

Fig. 1

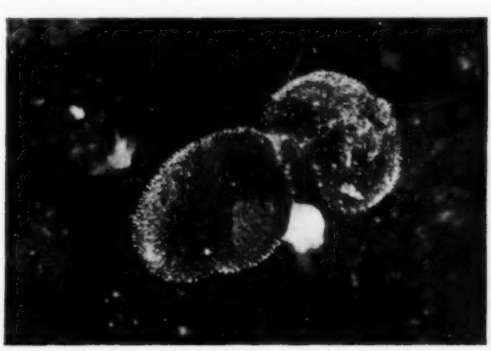

Fig. 2

syncotyly clearly formed by fusion of the normal ones and appearing as a single organ, often bent in various ways, such as with *Achillaea millefolium*, *Celsia* (Fig. 2), *Solidago virgaurea*, *Spiraea ulmaria*, *Tanacetum vulgare*. Only in two species, namely, *Angelica silvestris* and *Allium schoenoprasum* did seedlings occur which were entirely lacking in chlorophyll, or were of a more or less yellow-green colour.

As a rule, the true 3-cotyledons developed into plants with a leaf position which was other than that of the parent plants; thus plants originating from parents with opposing leaves became verticillate with three leaves in the whorl (*Kallanchoë*, *Lythrum*); again, those with scattered leaves mutated to an almost pseudo-verticillate leaf position (*Dimorphotheca*, *Nemophila*, *Centaurea* and *Aichryson*) with very densely scattered leaves, sometimes growing into giant individuals.

As is known, both polycotyledonous and monocotyledonous seedlings of a number of different dicotyledonic species have on occasion been found to occur spontaneously in Nature. Among my control cultures, only *Lythrum salicaria* showed a slight tendency to produce tricotyledons spontaneously; of 400 seedlings, one was found to have three cotyledons.

Fig. 3

However, some of the anomalous seedlings developed quite normally with no teratological characteristics, although the majority were subject to fasciations of various kinds. Most important and interesting was the occurrence of bud mutations, which sometimes appeared as early as at the seedling stage, but most commonly in more developed young plants. In the majority of cases they appeared as more or less strange fasciations with anomalous leaves and flowers. However, insufficient study has been devoted so far to the hereditary pattern of these 'thermomorphoses'. Apart from these teratological malformations in the progeny of the experimental plants, a number of other mutations also took place, both with plants and buds. In this category were included all individuals with strange characteristics which were observed neither in the parent population nor were known as existing in the species concerned elsewhere. So far as space permits, the following gives a survey of the most important cases investigated:

Seeds obtained from a burnt *Allium schoenoprasum* plant gave rise to 21 young plants, of which not a single one agreed in all its characteristics with the parent. The most marked peculiarities were discovered in seven of these plants; these displayed quite strange characteristics which had not been observed in the parent population nor had hitherto been known

Fig. 4

to occur elsewhere in any population of this species. In five of them, there developed among the flowers in the umbel bulbils which varied in number from few to many (Fig. 3), in a very similar fashion to *Allium oleraceum*; the remaining two became drooping umbels, as the single flower of *Galanthus*.

The results of experiments with *Angelica sylvestris* were no less surprising. Unfortunately only a small number of healthy seeds were obtained, and these gave rise to 26 seedlings. Even as early as in the first summer, the young plants showed many peculiar characteristics which were to be observed neither in the controls nor in the natural populations. One plant in particular was remarkable: as a seedling, it showed two plumules, the second of which sprouted from the root neck, obviously as a kind of bud mutation. The normal plumule developed common trifoliate leaves, but the bud mutation gave rise to only simple cuneate leaves (Fig. 4) tapering into a petiole with a broad apex irregularly toothed or incised, and thus bearing a strong resemblance to those of the Malesian *Trachymene koebrensis* and *T. novoguinëensis*. During the following summer, the same shoot had only pinnate leaves, with two to three pairs of leaflets, broadly ovate, serrate and somewhat convex, with some resemblance to the leaves of *Sium latifolium*. The main shoot gave rise to quite normal double pinnate leaves. In the third summer, this 'Siamese' twin plant reached the flowering stage. By then, the vegetative parts seemed to belong to two different plants, with a main stem 110 cm. high, pale green in colour, with double pin-

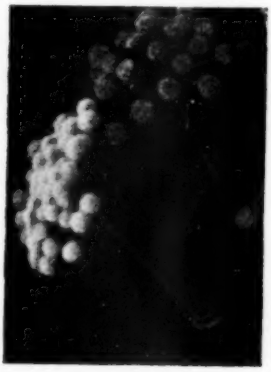

Fig. 5

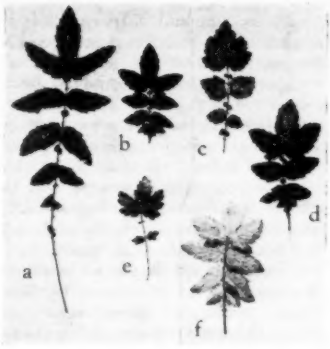

Fig. 6

nate *Angelica* leaves which were normal in every respect. The stem of the bud mutation grew only 70 cm., and was coarser, dark green in colour, with leaves which were broader and darker, with fewer and broader leaflets than those of the main stem. The umbels of the bud mutation were almost flattened, the umbelets depressed; rather few flowered, with flowers which were conspicuously larger than those of the parent population and of a milky white colour. The main stem had almost spherical umbels with dense globular umbelets and remarkably small, whitish, slightly purple-sprinkled flowers. The fruits of the main shoot were narrow lanceolate, approximately one-third longer than those of the parent, and of a vivid brownish shade. The mericarps of the bud shoot had a thick flattened corky rim instead of the normal thin membraneous one of the parent population, and had a greyish sooty colour.

These odd twins were crossed in both directions, but only the bud mutation gave viable seeds. Of 42, only 15 germinated, and these resulted in the same number of healthy young plants. Ten of these F_1 plants flowered last summer. They represented as many forms, not one of them resembling either the others or the individuals of the parent population. Some plants were strikingly different in appearance; they had not been observed before in any *Angelica silvestris* population known to me. Their mode of growth approached that of *Angelica littorialis* rather than that of the wild *Angelica*. One of them is worthy of special note: the plant itself was low, large and richly ramified, with short internodes and broad leaves, with conspicuous sheaths which were entirely milky white in colour. The lower part of the stem and the branches were also glossy white. The umbels (Fig. 5*a*) were large and very irregular in size, extremely dense, and composed of about twice as many umbelets as normally occur in any plant of the parent population (Fig. 5*b*); these were irregular and almost flat, bearing a closer resemblance to some Japanese *Angelica* species than to those in Finland. The umbelets were also conspicuously dense, hemispherical, and the flowers ivory white. Another similar plant had the white stem and leaf sheaths striped with purple.

No less surprising results were obtained from the experiments with *Spiraea ulmaria*. About 31 per cent of the young plants of the first summer were mutated in various ways; during the last, that is, the second summer, the mutations were more pronounced. Some could be termed giants (Fig. 6*a*), with the leaf attaining a length of 34 cm., and four pairs of large leaflets. Others displayed a smaller number of leaflet pairs, but had a prominent terminal leaflet, often of peculiar form, either circular or lobed, toothed, undulated or crispate (Fig. 6*b*, *c*, *d*), and in several instances with the leaves white-spotted or striated (Fig. 6*f*). One plant had a dwarfish growth, densely tufted, with numerous small delicate leaves with only one pair of small leaflets and a large terminal leaflet which strongly resembled the leaves of some *Ribes* species (Fig. 6*e*).

Generally speaking, all the species treated in this way had teratological progenies, which provided evidence for either mere malformations of different parts of the plant or more 'normal' alterations in the size of the leaves and in colour, structure and number of the various parts of the flower.

My experiments have shown that a common burning glass may have the same effect in producing mutations as the Röntgen tube and radium needle.

EFFECTIVENESS OF LIPID AND LIPIDOPHILIC SUBSTANCES AS ADJUVANTS

By D. W. DRESSER*

Department of Zoology, University of Edinburgh

FREUND[1] has stated that adjuvants of the Freund type may act in one or more of three ways: (1) by storing antigen at the site of injection and releasing it slowly; (2) by stimulating the migration of macrophages and lymphocytes to the injection site; (3) by stimulating antibody production in some way which is not understood. This preliminary article is a brief description of a method whereby the effectiveness of different substances as stimulants of antibody production can be tested. In these experiments the antigen and adjuvant have been injected by different routes so that Freund's categories (1) and (2) can be eliminated as the cause of the observed effect, for which the name 'adjuvanticity' seems to be suitable.

It has been known for some time that intravenous injection of protein solutions into rodents is an unreliable method of immunization. This has been confirmed[2] using BGG (bovine gamma globulin, Armour and Co.) in *CBA* strain mice. Intravenous injection of a freshly made solution in 0·9 per cent saline was found to immunize 5 out of 23 mice when the effectiveness of the first injection was tested a month later by the antigen-elimination technique[3] with BGG labelled with iodine-131 (BGG–^{131}I).

In some recent experiments, summarized in Table 1, it has been shown that no mice at all out of

* Present address: Division of Biology, California Institute of Technology, Pasadena, Calif., U.S.A.

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43 tested were immunized when the BGG solution first injected into the mice was centrifuged at 30,000*g* for 15 min. A very small amount of precipitate was always found after centrifugation, and it is thought that it is this material removed by centrifugation which enabled some of the mice injected with uncentrifuged BGG solution to be immunized. Freund's adjuvant containing BGG injected subcutaneously has been shown to be an effective means of immunizing mice. In Table 1 the secondary responses obtained after challenge with BGG–^{131}I, a month after the first antigen injection, have been arbitarily divided into five categories ranging from no response at all (=0) to the degree of response obtained after previous immunization with BGG in Freund's adjuvant (=4).

It was found that an intravenous injection of centrifuged BGG was effective in conferring immunity if a separate subcutaneous injection of Freund's adjuvant, made up without the antigen, was given at the same time. Several substances have been tried in place of the Freund's adjuvant without antigen. The results of these experiments are included in the table. Of the substances tested, 'Crill 16', oleic acid and powdered mycobacteria all have a marked adjuvanticity.

There seem to be two possible mechanisms responsible for adjuvanticity: (1) the adjuvant may affect the lymphoid cells of the animal, perhaps by stimulating them to take up the antigenic protein, or (2) the adjuvant, even when injected subcutaneously, may affect the stability of the circulating antigen, causing it to precipitate, thus immunizing the mouse in a similar way to alum-precipitated BGG. The latter alternative seems unlikely when the antigen is injected intravenously and the adjuvant subcutaneously, because a large part of the injected substances, for example, 'Crill 16', can be found 2 months later in subcutaneous vesicles. Although some of the 'Tweens' are known to affect the electrophoretic mobility of gamma globulins[4], it seems unlikely that they could affect the circulating BGG in preference to the large amounts of mouse protein present. Furthermore, 'Tween 60', although soluble in water and affecting gamma globulin, does not appear to have a marked adjuvanticity, whereas oleic acid is insoluble in water but is a good adjuvant. The first alternative, that the adjuvant affects the cells of the injected animal, seems to be the more likely.

Recent work has shown that mycobacterial waxes contain oleic acid, palmitic acid, various triglycerides and related substances[5]. It has been known for some time that mycobacterial waxes can be responsible for the establishment of hypersensitivity to complex antigens and simple chemicals[6]. A suspension of mycobacteria, oleic acid and some esters of sorbitol have been found to be substances with a marked adjuvanticity as measured by the method described here. Some of these esters (non-ionic detergents) are known to have deleterious effects[7], including carcinogenic effects[4], on animal tissues. It is possible that some lipids and non-ionic detergents, either in pure form or as mixtures in mycobacterial waxes or croton oil, may owe their adjuvanticity or carcinogenicity to their ability to damage cell membranes. In the first instance, adjuvanticity may be postulated as being due to a non-specific effect on cells, which might act in either or both of two ways: first, damaged cells may be stimulated to take up antigen by phagocytosis or pinocytosis; secondly, as a result of damage, lymphoid cells might be stimulated into a hypercompensatory reaction, one aspect of which could be the production of antibodies.

It seems that the observations recorded here may have a theoretical connexion with some auto-immune phenomena. BGG solutions centrifuged free of traces of particulate matter lack adjuvanticity in mice. When the BGG is particulate, for example after precipitation by alum, it possesses adjuvanticity on its own. It is not unlikely that most of the antigens with which an animal comes in contact during its life must have sufficient adjuvanticity either in themselves or closely associated with them for the immune mechanism to receive the necessary stimulus. There may, in fact, be two mechanisms preventing an animal from becoming immunized

Table 1

First injection of antigen	Treatment, 3 days before injection of antigen	Effect of challenge with 2 mgm. BGG-iodine-131 one month after first injection of antigen. Category of response, see text (No. mice responding—No. tested). (Increasing response →) 0	1	2	3	4
Freund's adjuvant containing 8 mgm. BGG/mouse; subcut.	Nil	—	—	—	—	25/25
5 mgm. alum-precipitated BGG; intraper.	Nil	—	—	—	—	5/5
5 mgm. BGG as sol. in 0·9 per cent saline; not centrifuged; intraven.	Nil	18/23	—	—	—	5/23
5 mgm. BGG as sol. in 0·9 per cent saline; centrifuged; intraven.	Nil	18/18	—	—	—	—
5 mgm. BGG as sol. in 0·9 per cent saline; centrifuged; intraper.	Nil	20/20	—	—	—	—
5 mgm. BGG as sol. in 0·9 per cent saline; centrifuged; subcut.	Nil	5/5	—	—	—	—
5 mgm. BGG as sol. in 0·9 per cent saline; centrifuged; intraper.	Freund's adjuvant made up with saline only; subcut.	—	—	—	—	10/10
5 mgm. BGG as sol. in 0·9 per cent saline; centrifuged; intraper.	2 mgm. powdered mycobacteria in saline; subcut.	—	—	—	—	5/5
5 mgm. BGG as sol. in 0·9 per cent saline; centrifuged; intraper.	0·4 ml. liquid paraffin; subcut.	9/10	—	—	1/10	—
5 mgm. BGG as sol. in 0·9 per cent saline; centrifuged; intraper.	0·4 ml. 'Crill 16'*; subcut.	—	—	—	—	13/13
5 mgm. BGG as sol. in 0·9 per cent saline; centrifuged; intraper.	0·2 ml. 'Tween 60'†; subcut.	5/5	—	—	—	—
5 mgm. BGG as sol. in 0·9 per cent saline; centrifuged; intraper.	0·2 ml. 'Span 20'‡; subcut.	1/5	—	—	1/5	3/5
5 mgm. BGG as sol. in 0·9 per cent saline; centrifuged; intraper.	0·2 ml. oleic acid; subcut.	—	—	1/5	—	4/5

* Commercial preparation of sorbitol monoleate containing other esters of sorbitol as impurities. Made by Croda, Ltd., Goole, Yorks.
† Polyoxyethylene sorbitol stearate. Made by Atlas Powder Co. (Honeywill and Stein, Ltd., Piccadilly, London).
‡ Sorbitol monolaurate. Made by Atlas Powder Co.

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against its own tissues; (1) lack of antigenicity (immunological tolerance); (2) lack of adjuvanticity. An example of the latter might be spinal cord or brain tissue. Allergic encephalomyelitis may in some cases be caused by prolonged injections of dried spinal cord tissue, but a single injection of spinal cord or brain in Freund's adjuvant will always lead to this condition[8]. This effect of Freund's adjuvant is unlikely to be due to the breakdown of a previously acquired state of tolerance, as mice tolerant to BGG do not give an immune response even when challenged by an injection of BGG in Freund's adjuvant[9]. It would seem from this that mammals are not normally immunologically tolerant to brain or spinal cord, and that the lack of an immune response is due to some other factor. It has recently been shown that tolerance to brain or spinal cord may possibly be induced experimentally[6]. It is a possibility, therefore, that this and some other auto-immune phenomena may be initiated by a gain of adjuvanticity by a tissue which normally lacks adjuvanticity, but to at least one antigen of which the animal is not normally immunologically tolerant.

I am grateful to Dr. R. A. Kille for the benefit of discussion.

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INCREASE OF THE RATE OF RELEASE OF THYROIDAL IODINE-131 AND OF CIRCULATING THYROTROPHIC ACTIVITY AT EARLY STAGES OF PROPYLTHIOURACIL TREATMENT IN THE RAT

By Dr. F. ESCOBAR del REY, G. MORREALE de ESCOBAR, M. D. GARCÍA GARCÍA and J. MOURIZ GARCÍA

Instituto G. Marañon, Consejo Superior de Investigaciones Científicas, Velázquez, 138, Madrid

THE idea that induction of goitrogenesis by propylthiouracil (PTU) and related compounds only involves those effects of such drugs which lead to the blocking of thyroid hormone synthesis is generally accepted. The hypothesis that these goitrogens do not have an extrathyroidal effect on thyroxine metabolism which might be playing a part in thyroid–pituitary interrelationships was confirmed in the case of thiourea when Griesbach and Purves[1] showed that thyroxine requirements for the maintenance of normal pituitary cytology of thyroidectomized animals were the same for control and treated rats. It has been widely assumed that this conclusion is valid for propylthiouracil also.

Observations have, however, been reported which are not in complete agreement with such hypotheses. Rawson[2] has observed a 50 per cent increase in the mean acinar cell height in rat thyroids as soon as 24 hr. after onset of PTU treatment, at which time no differences are yet demonstrable in the plasma protein-bound iodine. This finding was interpreted[2] as the result of an enhancing effect of PTU on thyrotrophic hormone (TSH) effectiveness[3,6]. Van Middlesworth[5] found that thyroxine doses necessary for suppression of PTU-induced goitrogenesis raised the plasma protein-bound iodine of the rat well above normal.

We have reported[6], moreover, that as soon as 5 hr. after onset of PTU administration to thyroidectomized, l-thyroxine-maintained rats, a depression of the peripheral deiodination of the hormone is demonstrable, this extrathyroidal effect of the drug lasting until suppression of treatment. The possibility was suggested that the peripheral effects of PTU which result in a decreased thyroxine dehalogenation might be triggering secretion of TSH by the pituitary, and that this response might be mediated by a factor related to the alteration of intracellular metabolism of thyroxine. Observations such as those summarized above[2,5] could then be interpreted along such lines.

This article reports an attempt to investigate the early phase of PTU treatment in the rat, when blocking of hormone synthesis by the drug has not yet resulted in a decreased plasma protein-bound iodine. Young adult male rats of a giant Wistar strain were used. They were fed a medium residue low-iodine diet. 10–15 μc. iodine-131 were injected intraperitoneally and the activity over the neck plotted against time. About 70 hr. after the injection of the tracer half the experimental group received 15 mgm. PTU intraperitoneally, and the radioactivity over the thyroid was followed for 24 hr., at which time all animals were killed. Fig. 1 shows typical results: immediately after PTU administration a sharp increase in the slope of the thyroidal iodine-131 disappearance curve has been consistently observed. These results do not exclude, among several possible interpretations, that the change in rate was due exclusively to blocking of iodine-131 recirculation by PTU. This possibility was investigated in a group of rats receiving 200 mgm./rat/day potassium perchlorate in order to avoid recirculation of radioiodide and thus measure the rate of release of labelled compounds from the thyroid. Half the group received later 2 × 10 mgm. PTU intraperitoneally, all animals being killed after 24 hr. As may be seen in Fig. 2, an increase of the thyroid iodine-131 release curve is observed soon after onset of PTU treatment. Due to the fact that the potassium perchlorate dose used is more than enough to block recirculation of iodine-131, the finding is interpreted as indicating a 100 per cent increase in the rate of secretion of labelled compounds from the gland. The protein-bound iodine-131 of the plasma was the same for both experimental groups or somewhat higher for PTU-treated rats. The histological appearance

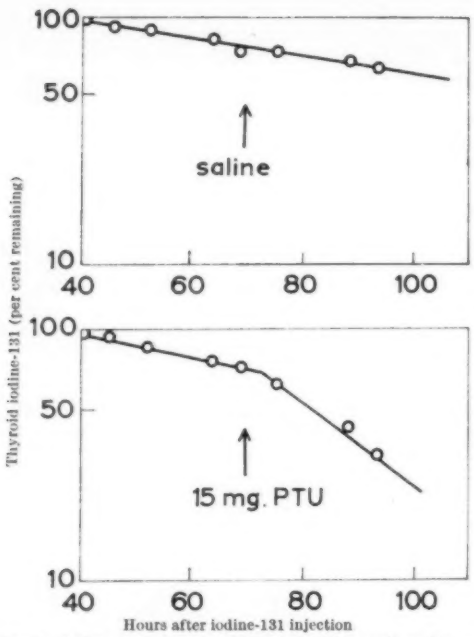

Fig. 1. Influence of 15 mgm. PTU injected intraperitoneally on the disappearance rate of iodine-131 from the thyroid, in male rats, injected with 10 μc. iodine-131. The radioactivity over the thyroid is expressed as percentage of the iodine-131 content 40 hr. after injection of the tracer; mean values of four animals in each group are given

of the thyroids of these PTU-treated animals showed clear signs of an increased activity when compared with their corresponding controls. The pooled plasmas of the animals the thyroidal data of which are shown in Fig. 2 were assayed for TSH activity by McKenzie's method[7]: the increase in blood radioactivity 2 hr. after intravenous injection of the sample is measured using mice injected with iodine-131, treated with thyroxine and thyroid powder to block endogenous TSH secretion. With 0·3 ml. of pooled plasma from the potassium perchlorate-treated controls the response was 119 ± 8 per cent (mean ± *S.E.*); with plasma from potassium perchlorate + PTU-treated animals it was 187 ± 15 per cent ($P < 0.01$). Injection of saline gives a response of about 95 per cent.

Two pools were made with plasmas from 4 male rats and with those from another group of animals injected 24 hr. previously with 15 mgm. PTU and assayed for TSH activity: the responses were 100 ± 6 per cent and 158 ± 5 per cent for control and PTU-treated rats respectively, $P < 0.001$. When 2 mgm. PTU were added per ml. of control plasma, the response was higher (130 ± 8 per cent) than that obtained without addition of the drug, though still significantly lower ($P = 0.01$) than that given by the plasma of PTU-treated animals. When the acetic acid (0·25 per cent)–saline extracts of the pooled pituitaries of these animals were tested for TSH activity, it was found to be significantly lower for PTU-treated animals as compared with controls. This finding was confirmed with pituitary extracts from two other experiments carried out as just described.

The thyroid iodine-131 release curve of perchlorate-treated rats receiving daily 2 × 20 μgm. 1-thyroxine to block endogenous TSH secretion was not altered by the administration of 2 × 10 mgm. PTU.

Since the hypotheses tested with the above experiments assumed that alterations in the peripheral metabolism of thyroxine induced by PTU might influence pituitary secretion of TSH, it was necessary to ascertain whether potassium perchlorate might be interfering with such extra-thyroidal effects of PTU or itself have an effect on peripheral thyroxine metabolism. On the basis of data reported by others[8] it seemed unlikely; however, the point was investigated in thyroidectomized, 1-thyroxine-maintained rats, isotopically equilibrated with radiothyroxine as described elsewhere[6]. Administration of 200 mgm./rat/day of potassium perchlorate alone or together with 15 mgm. PTU showed that perchlorate neither inhibits the peripheral deiodination of thyroxine nor interferes with the depressing effect of PTU.

As referred to above, Griesbach and Purves[1] found that 0·25 per cent thiourea in drinking water did not affect thyroxine requirements of thyroidectomized rats for the maintenance of normal pituitary cytology. Using the same experimental procedure indicated above we found that such doses of thiourea did not result in peripheral alterations of 1-thyroxine metabolism similar to those induced by PTU.

Some of the present findings are in good agreement with results recently reported by Mayberry and Astwood[9] while the present work was in progress: they found that the rate of disappearance of thyroid iodine-131 was greater for PTU-treated than for potassium perchlorate-treated rats. Moreover, a progressive disappearance of labelled thyroxine was observed from the hydrolysated thyroid of animals treated with PTU starting 24 hr. after injection of iodine-131. With either control, iodide- or perchlorate-treated animals, the proportion of thyroid radioactivity appearing as thyroxine remained unaltered during the seven-day observation period. This point is suggestive in view of the following hypotheses:

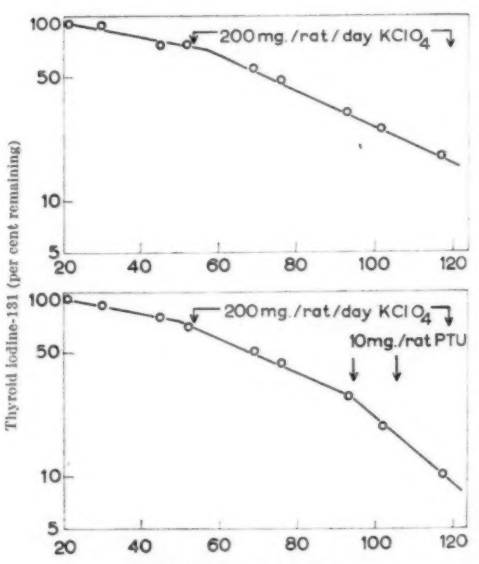

Fig. 2. Effect of PTU on the rate of release of iodine-131 labelled compounds from the thyroid of perchlorate-treated rats. Radioactivity over the thyroid is expressed as percentage of iodine-131 at 20 hr. after injection of 12 μc. Mean values of four animals in each group are given

We believe the present preliminary results may be tentatively interpreted as follows:

(*a*) At very early stages of treatment with PTU there is a considerable increase in the release of labelled compounds from the rat thyroid.

(*b*) Blocking of endogenous TSH secretion with thyroxine interferes with this effect of PTU.

(*c*) Within 24 hr. after onset of PTU treatment there is a higher thyrotrophic circulating activity than that corresponding to controls, as shown both by TSH bioassay of plasmas and thyroidal histology. The activity of pituitary TSH is lower, as shown by bioassay.

(*d*) The three points summarized above strongly suggest that PTU administration results very soon in an increased TSH secretion by the pituitary. Data reported by others[2,5,9] may be interpreted as leading to the same conclusion.

(*e*) It is further suggested that the rapid and intense alteration of the peripheral metabolism of thyroid hormone induced by PTU administration[6] plays an important part in this early response. Moreover, it might aggravate the effects of a low thyroxine pool in later stages of PTU treatment. The fact that blocking of endogenous TSH secretion abolished the effect of PTU makes a direct action of the drug on the pituitary or thyroid gland, not mediated via the thyroid hormone, rather unlikely. This finding does not disagree with the present interpretation: though PTU decreases the deiodination of such high doses of thyroxine to about half that of the corresponding controls (Escobar del Rey and Morreale de Escobar, unpublished results) the amount of thyroxine dehalogenated is still much higher than that corresponding to a physiological dose of the hormone. Neither do the findings of Griesbach and Purves[1] disagree with the present interpretation, since treatment with thiourea does not result in extrathyroidal alterations of thyroxine metabolism comparable to those exerted by PTU.

We should like to emphasize that the present interpretation is merely tentative and needs further experimental confirmation.

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TRANSFER FUNCTION OF A BIOLOGICAL PHOTORECEPTOR

By Dr. LAWRENCE STARK and Dr. HOWARD T. HERMANN

Neurology Section, Electronic Systems Laboratory, Massachusetts Institute of Technology, Cambridge, Massachusetts

A FULL description of the function of a sensory organ or sensory element should include definition of the limits of signal transfer. This can be done if the stimulating energy is monitored, and, at the same time, the nervous impulses emanating from the sensory receptor are detected and studied. It is believed that the signal which represents the input stimulus and which is transmitted to other centres of the central nervous system is the average frequency of these nerve impulses. By correlating the instantaneous values of the stimulus and the nerve impulse signal, it is possible to make a rigorous statement regarding the transfer characteristics of the sensory receptor. The task then is to find the equation (generally an integro-differential equation) which characterizes the functional relationship between input and output[1-4].

To explore the problem, we have chosen to study the photosensitive tail ganglion of *Cambarus astacus*, the common crayfish[5-8]. Behaviour and reflex studies have demonstrated a physiological role for the receptor[9-10].

Through a window in the ventral abdominal exoskeleton, large electrodes were applied to the ventral nerve cord. The animal was kept in a humidity- and temperature-controlled environment (100 per cent saturation, 19° ± 0·5° C.). An electronically controlled light source was used to generate transient steps as well as sinusoidal variations of light[11]. Relatively small light fluctuations in a darkened environment permitted small signal linearization.

The nerve impulses were averaged over a time period (*T.C.* = 0·24 sec.) long with respect to pulse-intervals, but short with respect to the response-frequency of the receptor. A combination of opera-

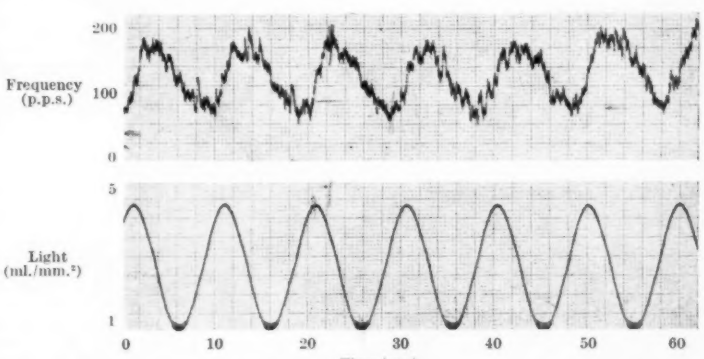

Fig. 1. Response of a photoreceptor to sinusoidal light changes. Note that the harmonic distortion is minimized by small signal approach. The 0·1 c.p.s. input produces an output with amplitude of ± 50 c.p.s., a mean of 130 p.p.s., and a phase-lag of 105°

tional amplifiers, pulse-shaping circuits, and low-pass filters carried out the averaging. An amplitude window screened from the averaging process any pulses with heights greater or smaller than the medium-sized pulses which carry the light energy information[12].

A response of the photoganglion to a sinusoidal stimulus is shown in Fig. 1. The noise seen comes in part from random groupings of pulses from different fibres. Harmonic distortion was minimized by the small-signal experimental conditions, but still is apparent here. The mean amplitude of response over a number of cycles and the mean phase relationship between stimulus and response were determined from such a recording. The parameter triplet—gain, phase and frequency—completely specifies a linear system.

The frequency response of the photosensitive ganglion was obtained by means of a series of experiments at different driving frequencies. Note in Fig. 2: the static or d.c. gain, the absence of adaptation, the break frequency, the attenuation slope, and the large phase lag, composed of both minimum and non-minimum elements. A transfer function:

$$G(s) = 32\,\frac{e^{-s}}{(1 + 1{\cdot}2s)^2}\ p \text{ sec.}^{-1} \text{ mlm.}^{-1}$$

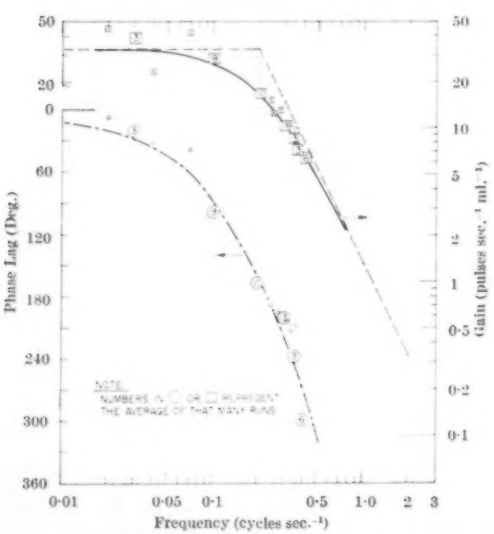

Fig. 2. Bode plot of photosensitive ganglion response. The solid curve represents the theoretical behaviour of a system described by the transfer function. The dashed lines are asymptotes showing the break frequency and attenuation slopes more clearly. The phase-lag curve is computed from the sum of minimum and non-minimum phase elements. The points are experimental

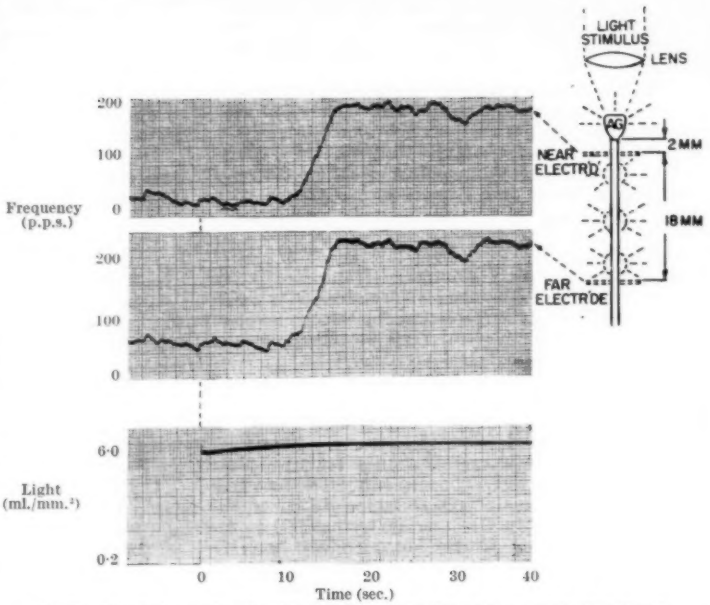

Fig. 3. Transient experiment. The lack of contribution of nerve conduction time to the non-minimum phase element (time delay) is shown. Thus, processes in the photosensitive ganglion are responsible for this operator. Conduction velocity is approximately 4 m./sec. It is interesting to note that details of the response are preserved over the 18 mm. conduction path, through three abdominal ganglia. This step of light was rather large, violating completely the theory of small perturbation analysis. Damping is inversely proportional to amplitude of forcing function

in Laplace operator ('s') notation can be quantitatively derived from this graphic display of system behaviour[3]. It is somewhat more convenient than the equivalent integro-differential equation:

$$32L(t - 1) = 1{\cdot}44\,\frac{d^2P}{dt^2} + 2{\cdot}4\,\frac{dP}{dt} + P$$

where L = light flux in millilumens, P = pulses per sec., t = time in sec.

The time-delay operator which accounts for the non-minimum phase lag is due, not to nerve conduction time, but rather to processes in the photosensitive ganglion, as reference to Fig. 3 demonstrates. Further investigations of non-linearities are proceeding; as can be seen in the large-signal step experiments, the law of superposition does not hold.

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DISTRIBUTION OF SODIUM AND POTASSIUM IN CERTAIN CELLS AND TISSUES

By Dr. NORMAN R. JOSEPH, Dr. MILTON B. ENGEL and Dr. HUBERT R. CATCHPOLE
University of Illinois Professional Colleges, Departments of Chemistry, Orthodontics and Pathology, Chicago

IN primitive oceans, as in the waters of all other epochs, the concentration of sodium greatly exceeds that of potassium, approaching in sea-water of the present era a ratio of about 45 : 1. An excess also occurs in the blood serum of vertebrates, where the ratio is about 30 : 1. In many cells and tissues, however, the ratio is much lower, reaching states in which potassium concentration is higher than sodium concentration. One method of explaining this apparent lack of equilibrium attempts to relate concentration gradients to intracellular metabolic processes; various 'pumps' and 'ion-carriers' deriving energy from respiration have been postulated. This view seems to have been accepted by biologists for many years, and has tended to become the dominant theory[1–3].

In our work with connective tissues, in which respiration is conceded to be low, it was found that ion distributions could be described in terms of the densities of negative colloidal charge and the standard free energies of ion-exchange reactions[4]. Changes in the charge densities of tissue surfaces in relation to *p*H have been represented as titration curves of the surfaces[5–7]. Similarly, Ling, working with nerve and muscle, has supported an explanation of ionic gradients in terms of fixed charges, ionic radii, and dielectric constants[8,9].

Extension of titration studies to frog muscle showed that when respiration was lowered by cooling the tissue, there was no important change in the titration curve (hence, in ion distribution), but that application of respiratory inhibitors greatly altered the curve by lowering colloidal charge[10]. This points to a possible fallacy in all experiments where respiratory poisons have been used to support the argument that ion distribution is determined by respiration rather than by colloidal state. Respiratory inhibitors such as fluoride and dinitrophenol are nucleophilic anions which are bound to amphoteric colloids (including respiratory enzymes), changing colloidal charge and ion distribution. In cooling experiments the effects of temperature and of anions are clearly separable. In attempts to demonstrate the effect of respiration on ion distribution, the possible simultaneous effects of inhibitors on the insoluble structural colloids have been considered only rarely[10].

Changes in Free Energy in Heterogeneous Systems

Cells and tissues are heterogeneous systems[11–14]. It is assumed therefore that they are composed of a limited number of phases, including colloid-rich and water-rich phases which are heterogeneous at submicroscopic levels, and which are in equilibrium with each other and with blood.

In the theoretical treatment to follow, the distribution of ions will be considered between blood serum, to be denoted phase 1, and a cellular or tissue phase, to be denoted phase 2. In accordance with the above definition, the properties of phase 2 should approach those of a heterogeneous aggregate rather than those of an isolable homogeneous phase. The following formulations apply to the equilibrium of sodium, potassium and chloride, but may be extended to include other mobile anions and cations. The theoretical method differs from that of Donnan[15] by introducing standard potentials of the ions in the two phases. When, for all the ions, the standard potentials are constant throughout the heterogeneous system, the resulting equilibrium conditions are those of the classical Donnan equilibrium. When the standard chemical potentials vary in different phases, the distribution ratios for sodium and potassium may be quite unequal at equilibrium. For the equilibrium of sodium and chloride in two phases:

$$Na' + Cl' = Na'' + Cl'' \qquad (1)$$

Table 1. VALUES OF Q_3 AND $-\Delta F_3^\circ$ FOR EXCHANGE REACTIONS $K' + Na'' = Na' + K''$ ($\Delta F_3^\circ = -RT \ln Q_3$)

State of aggregation	Low (or zero) metabolism (closed systems)					Moderate or high metabolism (open systems)				
	System		Q_3	$-\Delta F_3^\circ$ (kcal.)	Ref.	System		Q_3	$-\Delta F_3^\circ$ (kcal.)	Ref.
Solution	Œdema fluid	*a*	0·75	−0·17	19					
	Synovial fluid	*b*	0·8	−0·13	19					
	Ascitic fluid	*a*	1·0	0·0	19					
	Aqueous humour	*c*	1·2	0·10	20					
	Vitreous humour	*b*	1·5	0·24	20					
	Peritoneal fluid	*a*	0·85	−0·08	20					
	Thoracic lymph	*a*	0·95	−0·03	20					
	Blood dialysate	*d*	0·8	−0·13	19					
Solid or semi-solid	Zeolite			0·21	16	Peripheral nerve	*c*	5·5	1·0	20
	Zeolite			1·0	17	Kidney	*a*	18	1·75	20
	Dental enamel	*a*	3·6	0·8	20	Heart muscle	*g*	60	2·5	20
	Dentine	*a*	5·1	1·0	20	(A-V bundle)	*h*	17	1·7	27
	Bone	*a*	2·2	0·5	20	(Rt. ventricle)	*h*	57	2·5	27
	Erythrocyte	*a*	210	3·2	21	Whole brain	*d*	58	2·5	20
	Erythrocyte	*c*	260	3·4	21	Whole brain	*g*	67	2·6	20
	Erythrocyte	*d*	2·2	0·5	21	Whole brain	*f*	49	2·4	20
	Leucocyte	*c*	48	2·4	22	Whole brain	*i*	52	2·4	20
	Ascites cells	*e*	81	2·7	23	Skeletal muscle	*j*	111	2·9	28
	Whole skin	*a*	5·0	1·0	24	Uterus, muscle	*i*	29	2·1	29
	Epidermis	*a*	45	2·3	25	Liver	*i*	85	2·7	20
	Epidermis	*e*	36	2·2	26	Giant axon	*k*	90	2·7	30
	Cartilage	*f*	7·5	1·25	4					
	Lens	*a*	33	2·1	20					
	Lens	*b*	159	3·1	20					
	Lens	*f*	230	3·3	20					

a, Human; *b*, cattle; *c*, horse; *d*, dog; *e*, mouse; *f*, rabbit; *g*, guinea pig; *h*, ox; *i*, cat; *j*, rat; *k*, squid.

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where Na′ and Cl′ refer to phase 1, and Na″ and Cl″ refer to phase 2. Thermodynamic equilibrium is determined by the standard free-energy change, ΔF_1°, of reaction 1. A similar reaction describes the equilibrium of potassium and chloride:

$$K' + Cl' = K'' + Cl'' \quad (2)$$

Equilibrium in the system depends on six chemical potentials, one for each of the three ions, K, Na, and Cl, in each of the two phases. The chemical potential, $\bar{F}_{K'}$, of K′ in phase 1, is given by:

$$\bar{F}_{K'} = (\bar{F}_{K}^\circ)' + RT \ln (K') \quad (3)$$

where $(\bar{F}_{K}^\circ)'$ and (K′) are, respectively, the standard potential and concentration of the ion. (If equilibrium be considered for normal physiological states, it is convenient to define the standard chemical potential of an ion in any phase by equation 3. The activity coefficient referred to some other standard state (infinite dilution in water, for example) would be included in the standard chemical potential of the ion in the given phase. For such a 'physiological standard state' activity coefficients of sodium, potassium, and chloride would be 1·0 by definition, whereas the standard chemical potentials would be defined in terms of the ion concentrations at equilibrium.) Five additional equations of the same form are required for K″, Na′, Na″, Cl′ and Cl″. These yield the following expressions for ΔF_1 and ΔF_2, the free-energy changes of reactions 1 and 2:

$$\Delta F_1 = \Delta F_1^\circ + RT \ln \frac{(Na'') \times (Cl')}{(Na') \times (Cl')} \quad (4)$$

and:

$$\Delta F_2 = \Delta F_2^\circ + RT \ln \frac{(K'') \times (Cl'')}{(K') \times (Cl')} \quad (5)$$

where ΔF_1° and ΔF_2° are the standard free-energy changes. By combining equations 4 and 5, and eliminating the chloride ion:

$$\Delta F_3 = \Delta F_2^\circ + RT \ln \frac{(K'') \times (Na')}{(K') \times (Na'')} \quad (6)$$

where ΔF_3 and ΔF_3° refer to the reaction:

$$K' + Na'' = K'' + Na' \quad (7)$$

The free-energy changes are related as follows:

$$\Delta F_3 = \Delta F_2 - \Delta F_1 \quad (8)$$

$$\Delta F_3^\circ = \Delta F_2^\circ - \Delta F_1^\circ \quad (9)$$

The equilibrium conditions require that ΔF_1, ΔF_2 and ΔF_3 be zero. Then:

$$\Delta F_1^\circ = -RT \ln Q_1 \quad (10)$$

with two additional equations of the same form relating ΔF_2° to Q_2, and ΔF_3° to Q_3. The mass-law constants Q_1, Q_2 and Q_3 are defined by the ion ratios of equations 4, 5 and 6. They are therefore related by the formula:

$$Q_2 = Q_1 \times Q_3$$

Sodium–potassium equilibrium is expressed by Q_3:

$$Q_3 = \frac{(K'') \times (Na')}{(K') \times (Na'')}$$

When the standard free-energy change of reaction 7 is zero, $Q_3 = 1{\cdot}0$. When ΔF_1° and ΔF_2° are zero, Q_1 and Q_2 are also equal to 1·0. In this situation equilibrium is described by the classical Donnan conditions[15]:

$$\frac{(K'')}{(K')} = \frac{(Na'')}{(Na')} = \frac{(Cl')}{(Cl'')}$$

Otherwise the ratios Q_1, Q_2 and Q_3 depend on the three standard free-energy changes according to equation 10. From the four equations for the chemical potentials of K′, K″, Na′ and Na″, one obtains:

$$\Delta F_3^\circ = \Delta \bar{F}_{K}^\circ - \Delta \bar{F}_{Na}^\circ$$

The distribution of ions is therefore determined by the four standard potentials of the two ions in the two phases. The distribution of chloride is determined by the values of Q_1 and Q_2 (or by ΔF_1° and ΔF_2°). Any two values of Q_1, Q_2 or Q_3 are sufficient to determine the third. The equilibrium is then described by any two of the mass-action constants or standard free-energy changes, and by the colloidal charge density, which is usually negative[4,6]. Electrostatic equilibrium depends on the negative colloidal charge, x, which is balanced by mobile ions:

$$(Na'') + (K'') = (Cl'') + x$$

where it is assumed that other mobile ions are present in only small quantities. In the following discussion, sodium–potassium equilibrium is expressed independently of other ions in terms of calculated values of Q_3 and ΔF_3°. These values express 'potassium selectivity' according to the mass-action law. For any values of Q_3 and either Q_1 or Q_2, the equilibrium is then determined by the colloidal charge, x. These three quantities are sufficient to determine K″, Na″, and Cl″ from K′, Na′ and Cl′. In any heterogeneous system, therefore, equilibrium between blood serum and other phases depends on the colloidal charge of the given phase and the standard free-energy changes of ion-exchange reactions[4,6].

Values of Q_3 and ΔF_3° for a large number of systems are given (Table 1). These show a number of standard free-energy changes of 0·1 kcal. or less, indicating values of Q_3 near 1·0 and an approach to the ideal Donnan distribution of ions. This occurs in all systems in which the colloids are in aqueous solution. When water is the dispersion medium in both phases, $\Delta \bar{F}_{K}^\circ$ and $\Delta \bar{F}_{Na}^\circ$, the standard changes of chemical potential of potassium and sodium, tend to approach zero. For solid or semi-solid states of aggregation, on the other hand, ΔF_3° is negative, Q_3 is greater than 1·0, and potassium ions tend to accumulate in the colloidal phase. Increasing negative values of ΔF_3° indicate increasing accumulation of potassium ions in phase 2. This occurs not only in cells and tissues (Table 1) but also in solid structures such as zeolites[16,17].

Effects of Ionic Size and Hydration

In all systems the tendency of potassium to displace sodium may be at least partly explained by the relative effects of the ions on the diffuse double layer at the colloidal interface[16,18]. (The total surface area of the interface between the colloid-rich and water-rich phases of ground substance of connective tissue is of a high order of magnitude. Assuming most of the tissue water to be contained in the system of vacuoles described by Gersh *et al.*[12], the total surface area of the vacuoles would be about 60 m.² per cubic ml. of tissue water[21]. This estimate is based on diameters of 1000 Å. for the vacuoles and represents only extracellular active surface. Intracellular surface areas are probably of a similar high order of magnitude.) These effects are related to the 'average effective diameters' of the ions and to the dielectric constant of the solvent. The theory of Debye and Hückel yields the following expression for the

electrostatic potential ψ of a univalent cation with unit charge, ε:

$$\psi = \psi^0 - \frac{\varepsilon\varkappa}{D(1 + \varkappa a)}$$

where a is the 'average effective diameter' of the ions in an electrolyte solution; $\varkappa$ is proportional to the square root of the ionic strength, and is the reciprocal of the thickness of the ionic atmosphere in centimetres; D is the dielectric constant; ψ^0 is the potential at the surface of the ion in a solution at zero ionic strength. For a univalent ion of radius b:

$$\psi^0 = \frac{\varepsilon}{Db}$$

The standard chemical potentials of ions, $\bar{F}_{K}^{\circ}$ and $\bar{F}_{Na}^{\circ}$, depend on values of b, while the activity coefficients depend on a. Values of a and b for potassium and sodium in water are given in Table 2, with the free energies of hydration, which depend on b. In this treatment, ionic size terms are considered in relation to thermodynamic functions which determine equilibrium. These terms may also be related to rates of diffusion and exchange, as studied by Ling[9] and by Simon[12].

For the exchange reaction:

$$K(H_2O) + Na^{\prime\prime} = K^{\prime\prime} + Na(H_2O)$$

the standard free-energy change is $-16{\cdot}2$ kcal. (Table 2). This represents a theoretical limit for exchange reactions involving hydrated ions in an aqueous phase and a co-existent phase of low dielectric constant, in which some of the ions are not hydrated.

Table 2. Free Energies of Hydration of Sodium and Potassium (ref. 16)

Ion	Ionic radius (Å.) (crystal.) b	Ionic diameter (Å.) (av. effective) a	$-\Delta F^{\circ}$ (hydr.) (kcal.)
Na	0·98	5·2	89·7
K	1·33	4·1	73·5
Difference	—	—	16·2

Relation of Hydration to Entropy

Because of the lower hydration energy of potassium ions, the fraction of unhydrated ions in any phase would be much greater than for sodium. For this reason it would be possible for potassium to participate more readily in short-range electrostatic interactions in media of low dielectric constant. This implies a greater number of possible states of potassium ions than of sodium ions in the colloidal phase. The entropy of physicochemical systems increases with statistical probabilities of given states; for systems in which sodium and potassium are free to exchange, the negative free-energy change, ΔF_3, occurring for any spontaneous process, would be associated with a corresponding positive entropy change, ΔS_3. Because the free energy of hydration of sodium is greater than that of potassium, the standard entropy change, ΔS_3°, of reaction 7 would be positive. The reaction tends toward an equilibrium which yields the maximal number of ionic states, including all the interactive forms of unhydrated ions in the colloid-rich phase. This implies for all possible distributions of sodium and potassium an equilibrium state in which free energy is minimal and entropy maximal. The temperature coefficient of the ion distribution ratio, Q_3, and the temperature coefficient of the standard free-energy change depend on ΔS_3°. In particular:

$$\frac{\partial \Delta F_3^{\circ}}{\partial T} = -\Delta S_3^{\circ}$$

Since in general ΔS_3° would be positive, ΔF_3° would become increasingly negative as the temperature increases. If Q_3 is increased the equilibrium would shift towards potassium accumulation in the colloidal phase. When this is found experimentally, the usual interpretation has been to attribute the increase of potassium to increased metabolic rates. The effect of temperature on the distribution of potassium in erythrocytes has been interpreted in this way[2]. This ignores the possibility that the effect depends on the free energies of hydration of the ions. Potassium, having the lower free energy of hydration, has a greater tendency than sodium to form unhydrated ions in a colloid-rich phase; it therefore has more freedom to neutralize fixed charges[9,13]. The presence of hydrophobic charged colloids decreases the number of hydrated ions in any system and increases the entropies of all ions of the colloid-rich phase. This effect is selective for potassium. In all systems for which this is true, ΔS_3° of reaction 7 would be positive, and ΔF_3° negative.

Standard Free Energy and Ion Concentration Gradients

The values of ΔF_3° in Table 1 range from 0·17 kcal. to about -3 kcal. Values near zero indicate an approach to the ideal Donnan distribution, which occurs in body fluids with colloids in solution. For solid or semi-solid states of aggregation, the values range from about $-0{\cdot}2$ kcal. (zeolite or femur) up to -3 kcal. High negative values are found for erythrocytes, ascites cells, leucocytes, epidermis and lens. The most actively metabolizing tissues such as muscle and nerve show standard free-energy changes in the same range (-2 to -3 kcal.). Changes of sodium and potassium distribution which occur during physiological activity may depend on small transitory changes of the colloids following stimulation, as observed by light-scattering[32]. Changes of ΔF_3° of a few tenths kcal. would produce rather large shifts of sodium and potassium independently of mechanisms of ion-transport. Values of ΔF_3° in all systems depend on states of aggregation of cells and tissues and on the nature of the colloidal components such as proteins, glycoproteins, phospholipids and nucleoproteins.

Accordingly, it is unnecessary to invoke metabolic energy to explain steady concentration gradients in tissues. Distribution of anions and cations in steady states depends only on the colloidal charge and on the standard free energies of ion-exchange reactions[4,6]. Homeostasis within heterogeneous systems of cells and tissues depends on the maintenance of constant chemical potentials of water and electrolytes[4,14]. Examples of analogous concentration gradients at minimal free energy are very numerous, and would include the distributions of surface-active substances according to their adsorption isotherms, the distribution of gases in the gravitational field of the Earth according to molecular weights (law of atmospheres), and the distribution of ions in immiscible solvents. Concentration gradients in tissues depend on the standard potentials of the ions in different phases. These values depend on dielectric constants and on the ionic radii (b values). The presence of fixed negative colloidal charges establishes electrostatic

conditions for equilibrium, requiring an excess of mobile cations over mobile anions for electrical neutrality. The ratio of potassium to sodium at any charged interface depends on the 'average effective diameters' (a values). The distribution of cations depends on the distribution of colloidal charge, on the dielectric constants of various phases, and on hydration energies and sizes of the ions. Redistribution of ions during metabolic changes would depend on changes of colloidal charge and states of aggregation, on changes of concentration of various metabolites (lactic acid, carbon dioxide, di- and tri-carboxylic acids), and on redistributions of water accompanying these changes[31]. According to Donnan's treatment, ionic equilibrium is determined by the condition of minimal free energy. This condition is of general validity for cells and extracellular substances, but the standard chemical potentials of mobile ions in different phases of the heterogeneous system must be included in the formulation.

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DISTRIBUTION OF HUMAN CHROMOSOME COUNTS IN RELATION TO AGE

By PATRICIA A. JACOBS and W. M. COURT BROWN, O.B.E.

Medical Research Council Clinical Effects of Radiation Research Unit, Western General Hospital, Edinburgh

AND

Dr. RICHARD DOLL

Medical Research Council Statistical Research Unit, London School of Hygiene and Tropical Medicine, Gower Street, W.C.1

An investigation has been made of the distribution of chromosome counts in leucocytes from blood cultures, prepared by the technique of Moorhead *et al.*[1], with the final spreading of the chromosomes achieved by drying in air. Ninety-seven people (50 males and 47 females) have been examined who, on chromosome analysis, appeared to have a normal karyotype. These subjects ranged in age from 5 months to 82 years, and were drawn from volunteers, hospital patients and the parents of patients with chromosome abnormalities. No subject had received X-ray treatment, and none was known to have a malignant tumour. While it would have been preferable for the subjects to have been a sample of the general population, there is no reason for believing that the composition of the present group has introduced any serious bias into the findings.

Two other groups were also studied. One consisted of 24 phenotypic males, all with a diploid number of 47 and a complement of XXY sex chromosomes. These patients ranged in age from a new-born child to a man of 68 years. The other group consisted of 42 mongol imbeciles (26 males and 16 females), ranging in age from 3 to 51 years, and all shown to be trisomic for a small acrocentric autosome, presumed to be No. 21 (Denver Classification). There was no evidence that any patient with a chromosome abnormality was a chromosome mosaic.

For the 97 subjects with a normal karyotype, the distribution of chromosome counts has been examined in eight age-groups (Table 1). For the abnormal subjects, three age-groups were studied in the XXY males, and four in the mongol imbeciles (Table 2). An average of 36 cells was counted for each subject with a normal karyotype, 30 for each XXY male, and 32 for each mongol imbecile. Less than 25 cells were scored in only 4 normals, 2 XXY males and 2 mongol imbeciles. Variations from subject to subject in the number of cells scored were allowed for in calculating the average age of each age-group, by weighting the age for the number of cells counted.

The chromosome preparations were scanned under low-power for dividing cells in which the chromosomes were well spread. These were then examined under an oil-immersion lens to decide whether they were suitable for counting. Three criteria had to be satisfied. First, the cell must not be obviously broken; secondly, there must be no stray chromosomes in the same optical field; thirdly, the fixation and spreading of the chromosomes must be such that there is no ambiguity in interpretation for the purposes of counting. If any one of these criteria was not

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Table 1. Distribution of Chromosome Counts in Cells of Persons with a Normal Karyotype

Age-group (yr.)	No. of subjects	Mean age of subjects* (yr.)	Total No. of cells counted	No. of cells with chromosome counts of: < 43	43	44	45	46	47	48	> 48	Hypomodal counts as percentage of modal counts	Hypermodal counts as percentage of modal counts
0–4	8	1·49	222	1	0	2	9	209	1	0	0	5·74	0·48
5–14	8	9·78	276	0	0	0	8	265	3	0	0	3·02	1·13
15–24	26	21·53	1,087	2	2	4	36	1,036	6	1	0	4·25	0·68
25–34	16	28·91	626	0	3	2	19	595	6	1	0	4·03	1·18
35–44	14	38·23	450	0	0	1	20	420	9	0	0	5·00	2·14
45–54	6	49·09	276	1	0	0	17	254	4	0	0	7·09	1·57
55–64	9	57·77	271	0	3	0	13	251	2	2	0	6·37	1·59
65 or more	10	72·18	320	1	2	5	19	283	10	0	0	9·54	3·53
All ages	97	34·42	3,528	5	10	14	141	3,313	41	4	0	5·13	1·36

* Weighted for the number of cells counted at each age.

satisfied, the cell was discarded; once the chromosomes had been counted, however, the count was always included.

Not all cells that satisfy these criteria can be analysed precisely; in particular, full analysis is often impracticable if there is excessive contraction of the chromosomes, although an accurate count is still possible.

In all, there were 66 cells with hypermodal counts (Tables 1 and 2), including 45 from persons with a normal karyotype. Of the 66 cells, 6 could not be analysed because of excessive contraction of the chromosomes, and in 5 other cells the distribution of the chromosomes between the various sub-groups recognized in the Denver classification was grossly abnormal. Among the remaining 55 cells, there were 38 from persons with normal karyotypes, with a total of 43 additional chromosomes. After two telocentric chromosomes were excluded, it was found that there was a slight excess of chromosomes in the 13–22 range of the Denver classification (24 against 18·39 expected); but this excess was not statistically significant.

Occasionally chromatid fragments, chromosome fragments and telocentric chromosomes were found. Chromatid fragments were always in proximity to their parent chromosomes, and, therefore, were assumed to have arisen from breakage of a chromatid during the preparation of the slide. They were not scored as separate bodies. Chromosome fragments were of two types depending on whether or not the fragment lay close to the parent chromosome. In the latter instance, analysis of the cell usually revealed the chromosome from which the fragment was derived, and neither type was scored as a separate chromosome.

Telocentric chromosomes do not occur in the normal human karyotype, and their presence in a cell is taken to indicate the breakage of a chromosome transversely through its centromere. They were uncommon in our preparations, and, when present, were almost always in pairs. Analysis of such cells always showed a metacentric or a sub-metacentric chromosome to be missing, the breakage of which is presumed to have given rise to the two telocentrics. A cell with two telocentric chromosomes and a count of 47 bodies was therefore recorded as having 46 chromosomes. Very occasionally a single telocentric was present, and our present practice is to score this as a chromosome.

In Tables 1 and 2, the numbers of cells in persons in each age-group with counts (*a*) greater than the modal number, and (*b*) less than the modal number, have been expressed as percentages of the number of cells with the modal number of chromosomes. The rates of change with age are indicated by the coefficients of x in the following regression formulæ:

For the proportion of hypermodal cells in subjects with a normal karyotype:

$$y = 0{\cdot}034x + 0{\cdot}369 \qquad (1)$$

For the proportion of hypomodal cells in subjects with a normal karyotype:

$$y = 0{\cdot}066x + 3{\cdot}384 \qquad (2)$$

For the proportion of hypermodal cells in subjects with an abnormal karyotype:

$$y = 0{\cdot}057x - 0{\cdot}229 \qquad (3)$$

For the proportion of hypomodal cells in subjects with an abnormal karyotype:

$$y = 0{\cdot}097x + 3{\cdot}550 \qquad (4)$$

Three of the regression coefficients are significantly different from zero: for (1), $P < 0{\cdot}01$; (2), $P = 0{\cdot}03$; and (4), $P = 0{\cdot}01$. The remaining coefficient (3) is not significantly different from zero ($P = 0{\cdot}07$).

An analysis of variance for all the data shows that the differences between the regression coefficients, considered as a group, can reasonably be attributed to chance ($P = 0{\cdot}2$), and the same result is obtained by examining separately the differences between the regression coefficients for the hypermodal and

Table 2. Distribution of Chromosome Counts among the Cells of Patients with Klinefelter's Syndrome or Mongolism

Diagnosis	Age-group (yr.)	No. of subjects	Mean age of subjects* (yr.)	Total No. of cells counted	No. of cells with chromosome counts of: < 44	44	45	46	47	48	49	> 49	Hypomodal counts as percentage of modal counts	Hypermodal counts as percentage of modal counts
Klinefelter's syndrome	0–24	9	12·16	279	0	0	3	10	266	0	0	0	4·89	0·00
	25–34	8	28·91	237	2	0	4	9	219	3	0	0	6·85	1·36
	35 or more	7	47·59	206	0	0	3	10	187	4	1	1	6·95	3·21
	All ages	24	27·77	722	2	0	10	29	672	7	1	1	6·10	1·34
Mongolism	0–14	12	9·69	393	1	0	2	11	376	3	0	0	3·72	0·80
	15–24	13	16·75	396	2	0	6	12	374	2	0	0	5·35	0·53
	25–34	6	29·95	232	0	0	2	12	213	4	1	0	6·57	2·35
	35 or more	11	40·24	331	0	2	8	16	303	2	0	0	8·58	0·66
	All ages	42	22·74	1,352	3	2	18	51	1,266	11	1	0	5·85	0·95

* Weighted for the number of cells counted at each age.

hypomodal counts, and for the normal and abnormal karyotypes.

It may be noted, however, that the rate of increase with age is greater for the hypomodal counts than for the hypermodal, and for the abnormal karyotypes than for the normal. The present data are not numerous, and the fact that the differences between the regression coefficients are not statistically significant does not necessarily mean that they are not real.

When plotted, the regression lines for the proportion of hypermodal cells (equations 1 and 3) pass close to the origin; in neither case is the regression constant significantly different from zero, and it would appear that at birth hypermodal cells must be rare. In contrast, the constants in the regression formulæ for the proportion of hypomodal cells (equations 2 and 4) are both significantly different from zero (in each case, $P < 0{\cdot}01$). The two estimates of the proportion of hypomodal cells at 0 years of age are similar to one another (3·384 and 3·550 per cent, respectively), and it is reasonable to assume that, with the present technique, the ratio of hypomodal to modal cells at birth would be about 3·5 per cent.

It has been assumed in the above analysis that the data are best fitted by straight lines. In the present limited state of knowledge this seems reasonable; in only one case (hypomodal cells in persons with a normal karyotype) is there statistical evidence of a significantly better fit with a quadratic curve.

One explanation for these findings could be that all non-modal cells are artefacts arising during preparation. A study of the hypermodal cells, however, provides evidence that this is not so. The type of artefact which will give rise to non-modal counts is rupture of a cell with the loss of one or more chromosomes; if these lost chromosomes become included in a neighbouring cell, a hypermodal count can be obtained. Usually, however, a true hypermodal cell can be distinguished from one which has gained a chromosome from a neighbouring cell. Such a 'foreign' chromosome can be recognized, because the degree of contraction of the chromosomes in a preparation tends to differ between cells, as the cells will be in different stages of their division cycle when colchicine is added to the culture medium. A further type of error which can give rise to a count of 47 is the presence of a single telocentric chromosome. As noted, our present practice is to record such cells as having a genuine count of 47 chromosomes. This may be wrong, but the number of cells involved is very small.

The great majority of the cells with hypermodal counts are therefore regarded as the products of errors in division. One possible mechanism by which these cells could be produced is somatic non-disjunction in a normal diploid cell. There will, of course, be an equal number of cells formed with counts of 45 chromosomes, and, if viable, these will be represented among the hypomodal cells. Hypomodal cells are also likely to be produced by anaphase lagging. The analysis of hypomodal cells, however, cannot distinguish between those that result from artefacts and those that are the products of errors in division. That the latter do occur can be inferred only from the study of the hypermodal cells. It follows, in our view, that the presence of aneuploid cells cannot be accounted for wholly on the basis of artefacts, nor can they all be regarded as genuine aberrations. It is suggested that the figure of 3·5 per cent hypomodal cells at birth, calculated from the regression formulæ, may provide an approximate estimate of the proportion of aneuploid cells arising from artefacts with the present technique.

Several biological mechanisms could be postulated to account for these results. One is that there is an increase in the frequency of abnormal divisions with age. These abnormal divisions may have occurred in ancestral cells, so that the cells which divide in culture to produce abnormal daughter cells were themselves abnormal when taken from the body. Another mechanism could be that the rate of abnormal division is a constant; but that some of the abnormal daughter cells are viable and maintain themselves in equilibrium with the normal cells. Lastly, it may be that the cells were normal when taken from the body and that abnormal division is related to the conditions of culture. In either case there exists some age factor which leads to the presence in the cultures of more abnormal cells in an older subject than in a younger subject.

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RECOMBINATION IN THE *X*-CHROMOSOME OF *DROSOPHILA MELANOGASTER*

By C. N. LAW*

Department of Agricultural Botany, University College of Wales, Aberystwyth

PREVIOUS work on *Drosophila* species has indicated that inter-chromosomal effects and genetic differences between the homologous chromosomes being studied influence recombination appreciably[1,2]. So far, few attempts have been made to determine whether or not interactions are occurring at the intra-chromosomal level. Recently, an experiment using different wild-type iso-alleles at the white-eye locus (*w*) has suggested that the presence of different alleles of major genes may influence recombination in their own vicinity[3]. In view of this, experiments have been carried out to investigate more closely the inter-relations between genes and regions within a chromosome.

Recombination was studied in an *X* chromosome, carrying the genes, yellow Hairy-wing (*yHw*), vermilion eye (*v*) and Bar-eye (*B*). The positions of these genes as indicated by Bridges are respectively, 0·0, 33·0 and 57·0. (For the purpose of this experiment, *y* and *Hw* are considered to be one gene, since they are both situated at position 0·0.) An inbred Samarkand–Bayfordbury stock was used, into which, by the use of a *CyL*/*Pm*; *Sb*/*H* technique, a single marked *X* chromosome was introduced without any appreciable alteration to the other chromosomes.

This stock was developed in such a way that all informative combinations of homozygosity and heterozygosity for two alleles at the three loci could be derived simultaneously (Fig. 1). Thus the experi-

* Present address: Plant Breeding Institute, Trumpington, Cambridge.

Fig. 1. Experimental design

$\frac{yHw\ v\ B}{+\ +\ +}$	$\frac{yHw\ +\ B}{+\ +\ +}$	$\frac{yHw\ v\ +}{+\ +\ +}$	$\frac{+\ v\ B}{+\ +\ +}$
$\frac{yHw\ v\ +}{+\ +\ B}$	$\frac{yHw\ +\ +}{+\ +\ B}$	$\frac{yHw\ +\ +}{+\ v\ +}$	$\frac{+\ v\ +}{+\ +\ B}$
$\frac{yHw\ +\ +}{+\ v\ B}$	$\frac{yHw\ v\ +}{+\ v\ B}$	$\frac{yHw\ +\ B}{+\ v\ B}$	$\frac{yHw\ v\ B}{yHw\ +\ +}$
$\frac{yHw\ +\ B}{+\ v\ +}$	$\frac{yHw\ v\ B}{+\ v\ +}$	$\frac{yHw\ v\ B}{+\ +\ B}$	$\frac{yHw\ v\ +}{yHw\ +\ B}$

ment permits the study of the influence of B and the proximal region on recombination distally, yHw-v; the effect of the median region containing the gene v on the whole marked region, yHw-B; and the effect of yHw and the distal region on the proximal segment, v-B. The experiment was carried out at 24° C., and recombination was estimated by the product formula.

Table 1 shows that different states of the distal region have insignificant effects on recombination in the proximal region, v-B, ($\chi^2_{(2)} = 0{\cdot}3414$, $P = 0{\cdot}90$–$0{\cdot}80$). The other two regions, however, have large influences on recombination. Thus, there are significant differences in recombination in the distal segment, yHw-v, when the different states of the B locus and the proximal region are considered ($\chi^2_{(2)} = 9{\cdot}2297$, $P < 0{\cdot}01$). Similarly, differences in the median region with the v locus are associated with significant effects on recombination for the whole chromosome, yHw-B ($\chi^2_{(2)} = 10{\cdot}7384$, $P < 0{\cdot}01$).

Table 1. Progeny obtained from Coupling and Repulsion Crosses for the Nine Parental Genotypes

Region	Parental genotype	Progeny				Recombination (ρ)
		Coupling cross		Repulsion cross		
		Recombinants	Total	Recombinants	Total	
Proximal	B/B	84	324	151	513	$0{\cdot}2765 \pm 0{\cdot}0160$
	$B/+$	489	1,827	540	2,001	$0{\cdot}2639 \pm 0{\cdot}0065$
	$+/+$	303	1,344	256	1,003	$0{\cdot}2400 \pm 0{\cdot}0097$
Median	v/v	352	912	449	1,192	$0{\cdot}3813 \pm 0{\cdot}0107$
	$v/+$	829	2,017	774	1,811	$0{\cdot}4192 \pm 0{\cdot}0088$
	$+/+$	350	766	464	1,144	$0{\cdot}4311 \pm 0{\cdot}0116$
Distal	yHw/yHw	92	464	54	302	$0{\cdot}1844 \pm 0{\cdot}0143$
	$yHw/+$	419	2,184	335	1,644	$0{\cdot}1978 \pm 0{\cdot}0065$
	$+/+$	281	1,382	122	638	$0{\cdot}1971 \pm 0{\cdot}0097$

The failure to detect an influence of the distal region on recombination, in contrast to the large effects produced by the other regions studied, may have a number of causes of which the following are considered here. Either the proximal and median regions are more active genetically than the distal region so far as the control of recombination is concerned, or the proximal and median regions are more active because of their position in relation to the centromere. Thus, if the recombination process commences proximally and moves towards the ends of the chromosome, and there is evidence to support this in *Drosophila*[4], direct genetic alteration of recombination in regions near the centromere must have consequences on recombination distally. On the other hand, direct genetic changes in distal recombination will have little effect proximally, because recombination will already have occurred here. Clearly, a definite distinction between these possibilities cannot be derived from the results obtained in these experiments. It seems, however, unlikely from examinations of other characters that the proximal and median regions should have a higher genetic activity than the distal region. The distributions of both genes controlling sternopleural chaetæ[5], and major genes in the X chromosome, are concentrated at both ends of the genetic map, so that according to such evidence the distal as well as the proximal region should show high activity. The most plausible hypothesis, therefore, appears to be the latter.

From the results it is also apparent that large variations in recombination are produced by the different genetic states used in the proximal and median regions. The possibility that some of this variation may arise as a result of the activity of the major genes used to measure recombination has, therefore, to be considered. A means of detecting such influences may be obtained from a comparison of the dominance relations expected from the normal phenotypic expressions of the genes involved and their observed level of action in recombination. Some agreement between the two may indicate that the major genes are having an effect. Evidence from the two major genes B and v, which lie in the regions producing significant effects on recombination, shows some agreement with expectation. Thus v acts as a recessive on eye-colour and appears to do likewise when recombination is considered, since a comparison between the homozygous recessive (v/v) and the combined homozygous and heterozygous dominant ($+/+$ and $v/+$), accounts for almost all the heterogeneity in this region and is highly significant ($\chi^2_{(1)} = 10{\cdot}4483$, $P < 0{\cdot}001$). Similarly, the gene B, which behaves as a partial dominant on eye-shape, produces analogous effects on recombination. In this case, the difference between the homozygous recessive ($+/+$) and the combined homozygous and heterozygous dominant (B/B and $B/+$) is also significant ($\chi^2_{(1)} = 8{\cdot}7194$. $P < 0{\cdot}01$). It appears, therefore, that some of the results could be produced by the major genes themselves and not entirely from genes closely linked to them.

The observations made in these experiments clearly demonstrate that large differences in recombination can be obtained by genetic alterations within a chromosome. Large structural changes seem to be excluded, since the combinatorial type of experimental design used here should show disturbance of this type. The lack of precision brought about by the use of large genetic map distances, which of necessity must be used in *Drosophila*, cannot allow an exact appraisal of the genetic factors responsible. Some of the differences may, however, be produced by the major genes used for estimating recombination. Further, the experiments indicate a definite relationship between regions and, moreover, one which appears to be orientated with respect to the centromere. They also illustrate the possible usefulness of such designs in recombination studies within a chromosome, particularly where small regions of a chromosome could be considered.

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XUM

LETTERS TO THE EDITORS

ASTROPHYSICS

Terrestrial Accretion from the Solar Wind

C. M. de Turville[1] has suggested that most of the water now present on Earth has been formed from hydrogen in the solar wind, in combination with oxygen in the Earth's atmosphere. If this is so, then the process must still be going on, forming water vapour, according to de Turville, at a rate of about 2×10^{-12} gm. cm.$^{-2}$ sec.$^{-1}$. The water is presumably being formed at very high levels (above 100 km.) where oxygen in the atomic form exists. The continuous formation of water vapour at these levels must lead to a gradient of the water vapour mixing ratio (the ratio of the mass of water vapour to the mass of air) decreasing downwards until overtaken by the gradient decreasing upwards arising from evaporation of water from the surface of the Earth. What measurements there are do suggest such gradients[2], and it is interesting to consider whether the humidities found could conceivably arise in this way.

We may assume a flux equation of the form $F = K\rho \frac{d\chi}{dt}$, where χ is the mixing ratio. If we now assume that K is invariant with height, and assume that the air density, ρ, is given by the equation $\rho = \rho_0(\exp -bz)$, then we find, inserting an appropriate value for b, and putting F equal to 2×10^{-12}:

$$\chi - \chi_0 = \frac{1{\cdot}5 \times 10^{-6}}{K}\left(\frac{1}{\rho} - \frac{1}{\rho_0}\right) \qquad (1)$$

or, if m denotes the water vapour density:

$$m = \frac{1{\cdot}5 \times 10^{-6}}{K}\left(1 - \frac{\rho}{\rho_0}\right) + \frac{\rho}{\rho_0} m \qquad (2)$$

It will be seen that, in any layer with a more or less constant K, the water vapour density tends rapidly to the value $\frac{1{\cdot}5 \times 10^{-6}}{K}$ gm. cm.$^{-3}$. Frost points corresponding to values of K ranging from 10^2 to 10^5 are given in Table 1.

Table 1

K	10^2	10^3	10^4	10^5
Frost points	−58	−74	−88	−101° C.
	215	199	185	172° K.

Few measurements of water vapour have been made at high levels, but Barclay *et al.*[3] obtained a value at 27 km., which corresponds to a frost point of −75° C. 'Mother-of-pearl' clouds are also occasionally seen, from Sweden, at these levels[4]. There is good reason for believing that these are water clouds, and since the temperature at these levels is known to be between −70° and −80° C., this also suggests a frost point of about −75° C. A flux of water vapour of 2×10^{-12} gm. cm.$^{-2}$ sec.$^{-1}$, and a turbulence coefficient of 10^3 would provide just such a frost point. This is rather a low value, even in this region of great stability, but it is certainly not so low that it can be dismissed as inconceivable.

There are no measurements of humidity at greater heights, but clouds—'noctilucent clouds'—are also reported, from high latitudes in summer, at levels of about 80 km. These look very much like water (ice) clouds, but the great difficulty, hitherto, in explaining how the water got there has led some meteorologists to conclude that they must be dust clouds[4]. The temperature at this level, in the regions where the clouds are seen, has never been measured, but it is probable that it is, in summer, very low—perhaps −90° C. or lower. This suggests a value for the turbulence coefficient of between 10^4 and 10^5—and this higher value is consistent with the known instability in this region (temperature maximum at about 55 km. and temperature minimum at about 85 km.). Some spectroscopic measurements in a water vapour absorption band have been made from aircraft[5]. Unfortunately, the absorption coefficients, at the wave-lengths used, are dependent on pressure, so that it is not possible to deduce the amount of water vapour above the level of the highest reading; but a rough calculation suggests that the results of Houghton and Seeley are quite consistent with the amounts suggested here.

It must be concluded that if water vapour is being added at the rate suggested by de Turville, then water (ice) clouds at about 80 km. and at about 25 km., and such measurements of water vapour at these levels as have been made, are all consistent with turbulent diffusion downwards of this water vapour, and coefficients of diffusion which it is reasonable to suppose may be found at the levels concerned.

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Ronald Frith

Meteorological Office,
Air Ministry,
London.

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de Turville[1] has suggested that the Earth is gaining hydrogen from the Sun at the rate of 1·5 tons a second, and points out that this rate of accretion will account for all the hydrogen in the oceans in the course of 3×10^9 years. His idea is that the geomagnetic field captures the protons of an incident solar wind.

Pirie[2] has expressed some concern at the idea of a 'hydrogen hurricane' depositing hydrogen in the atmosphere at such a high rate. For if de Turville is correct, the question is where does the oxygen come from? The suggestion therefore creates rather than solves a problem.

The values proposed by de Turville, a density of $n = 12$ protons cm.$^{-3}$ and a velocity of $v = 4{,}300$ km. sec.$^{-1}$, have indeed transformed the solar wind into a 'hydrogen hurricane'. These values yield a particle energy flux of 10^3 ergs cm.^{2} sec.$^{-1}$ at the Earth's orbit, or one-thousandth of all the radiation flux from the Sun. Where does this immense amount of energy

come from? It is assumed apparently that the particles are continuously accelerated as they travel from the Sun to the Earth, but it is not explained how this is possible. Parker, who is the principal advocate of the solar wind idea, suggests that hydromagnetic waves heat the solar corona out to a distance of possibly 8 solar radii, and therefore an isothermal corona must expand hydrodynamically away from the Sun. Beyond 8 solar radii the solar wind flows at a constant velocity, and the temperature falls off adiabatically. Applying de Turville's relation of $v \propto r^{2/3}$ out to a distance of only 8 solar radii, and not to the Earth's orbit, yields a solar wind velocity of 600 km. sec.$^{-1}$, in agreement with the more modest ideas that are prevalent.

Even if de Turville's values were correct, it is doubtful whether the hydrogen in the oceans can be accounted for in the manner he suggests. The momentum flux of this 'hydrogen hurricane' would deform and compress the geomagnetic field to within a distance of roughly 3 Earth radii, and not 12 Earth radii as stated. If the incident particles are captured by the geomagnetic field, the rate of accretion is reduced to only 5 per cent of the value proposed. However, one questions strongly whether the geomagnetic field does capture entirely the incident solar wind. In every analysis of the subject published so far, starting from the early work of Chapman and Ferraro, it is seen that the Earth's magnetic field acts as a shield, and forms a cavity inside the low-energy corpuscular streams from the Sun. The particles that penetrate, as a result of complex diffusion processes, are relatively few. If magnetic fields are embedded in the solar wind, and this is quite possible, the solar wind will stream hydrodynamically around and past the Earth, very much in the same manner that a fluid flows past a submerged object.

At the present moment it is not known definitely whether there is a solar wind or not. Should it turn out that there is a solar wind, or even a 'hydrogen hurricane', it is almost certain that the rate of accretion of hydrogen is negligible and cannot by any means account for the formation of the oceans in the life-time of the Earth.

E. R. Harrison

Atomic Energy Research Establishment,
Harwell,
Berks.

[1] de Turville, C. M., *Nature*, **190**, 156 (1961).
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Mr. Harrison has raised a number of important issues which can only be answered tentatively at the present time. Presumably the oxygen is liberated from terrestrial rocks by solar ultra-violet radiation, and an estimate of the mean quantum efficiency of this photochemical process should be readily obtainable in the laboratory, by irradiation of outgassed rock samples in an evacuated chamber, using a strong ultra-violet source and an ionization pressure gauge. In the absence of hydrogen accretion, this process would lead to an evolution of oxygen trending towards zero as ultra-violet absorption by atmospheric atomic and molecular oxygen and ozone increased, and the oxygen content of the atmosphere would reach asymptotically some terminal value corresponding to complete absorption of ultra-violet radiation. The effect of hydrogen accretion is to remove atomic oxygen at levels above 100 km., and the ultra-violet absorption provides a regulating mechanism which ensures a dynamic balance between the rate of liberation of oxygen from the rocks and the rate of removal of oxygen in the upper atmosphere.

Concerning the acceleration mechanism responsible for the solar wind, it must be understood that the difference between Parker's 600 km./sec. and the 4,300 km./sec. previously proposed by me is due solely to a different estimate of the range of applicability of the relation $v = Bn^{-\frac{1}{2}}$. I accept Parker's account of the origin of the solar wind in magneto-hydrodynamical terms, the ultimate source of energy being the thermal energy at the solar surface, which is clearly adequate. It is known from a study of the behaviour of solar cosmic rays that the region of disordered magnetic fields begins at about 1·2 A.U. from the Sun, and it seems plausible to suppose that the régime of flow of the solar wind is preserved until this point is reached. As pointed out by Parker[1], the assumption that the isothermal corona extends to only 8–20 $R_\odot$ and that beyond this point the temperature falls off adiabatically in the absence of any further coronal heating is merely the simplest and most conservative model consistent with observations of the intensity of electron scattering of photospheric white light; the fact that no observations are available beyond 20 $R_\odot$ is due to technical difficulties and affords no evidence of the absence of a corona beyond this point. If other evidence relating to conditions at the orbit of the Earth is also taken into account, Parker's estimates of the extent of the corona and of the velocity of corpuscular radiation turn out to be excessively conservative. The simplest model consistent with all observations is surely that of a radial solar wind the character of which is unaltered out to a distance of 1·2 A.U.

Nevertheless, it must be admitted that the numerical values previously quoted are seriously in need of revision, since the flight of *Pioneer V* has revealed that the velocity of the solar wind in the vicinity of the Earth is in fact $1{\cdot}45 \times 10^8$ cm. sec.$^{-1}$. Owing to equipartition of proton and electron energies in the terrestrial magnetic field, this implies a velocity $\sqrt{2}$ times larger in free space, so that the velocity of the solar wind in free space at the orbit of the Earth is $v_1 = 2{\cdot}05 \times 10^8$ cm. sec.$^{-1}$ and the corresponding ion concentration $n_1 = (B/v_1)^4 = 232$ cm.$^{-3}$, in better agreement with Biermann's estimate than with the values quoted by Pope and Kupperian—no theory can be expected to agree simultaneously with several discordant observations. The recalculated transit time of solar protons is then 2 days, but may well be shorter during magnetic storms. The rate of emission of hydrogen by the Sun becomes $2{\cdot}24 \times 10^{14}$ gm. sec.$^{-1}$, and implies a rate of emission of deuterium of $3{\cdot}3 \times 10^{11}$ deuterium atoms/cm.^{2} sec.

The adjusted values of n_1 and v_1 are quite compatible with van Allen's data[2] concerning the electronic energy and flux in the outer radiation belt. With the aid of these new values, it becomes possible to accept Harrison's valid objection concerning the deformation of the terrestrial magnetic field and yet recover the previous value of the rate of terrestrial accretion. The equatorial dipole field of the Earth is $H = 0{\cdot}316/x^3$ gauss, where x is the geocentric distance expressed in Earth radii, and if x_0 denotes the distance at which the pressure and magnetic energy density are equal, then $H^2/8\pi = n_1 m_H v_1^2/3$ and hence $x_0 = (80\pi n_1 m_H v_1^2/3)^{-1/6} = 3{\cdot}00$, which coincides approximately with the centre of the outer radiation belt.

XUM

The mass of hydrogen accreted by the Earth in time T is then $Q = \pi x_0^2 R_\oplus^2 m_H n_1 v_1 T$ and using these new values of x_0, n_1 and v_1 and the more plausible figure $T = 1{\cdot}5 \times 10^{17}$ sec. for the age of the Earth, the revised rate of accretion is $1{\cdot}37 \times 10^{23}$ gm., which is not very different from the previous value. Oxidation would yield $1{\cdot}23 \times 10^{24}$ gm. of water, but this is only a rough estimate. During magnetic storms, the rate of hydrogen capture may be greatly increased, and there are other complicating factors which indicate that the present simple treatment can only be accepted as a first approximation.

In particular, the question of the efficiency of capture and retention of particles from the solar wind is bound to be mathematically very complex, as it involves not only the combination of a radial solar magnetic field and a rotating terrestrial dipole field, but also the interaction of the incoming particles with the local plasma and the dissipation of electron energy by bremsstrahlung radiation. But if we are to judge by the recent findings discussed by Dr. Frith, the observational evidence afforded by high-altitude meteorology does suggest that the capture process is in fact fairly efficient. Thus the proposed account of the origin of the oceans remains eminently defensible, though much further work will be needed before it can be considered as established.

C. M. de Turville

35 Regent Street,
Clifton,
Bristol, 8.

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PHYSICS

Theory of the Red Shift and the Nature of Planck's Constant

Since the Mössbauer experiment, the definition of the frequency of light requires further attention. In the theory of General Relativity the following definitions are possible :

(1) *Based on Maxwell's Theory.* Let us call A_a (A_0, A_1, A_2, A_3) the four potential. Let us assume $A_a = a_a \rho^{i\varphi}$ as the solution of Maxwell's equations. The transition to geometrical optics follows from the assumption that $\left|\frac{\partial \varphi}{\partial x^a}\right| = \varphi_{/a}$ is very great. Maxwell's equations are then reduced to:

$$g^{\alpha\beta}\varphi_{/\alpha}\varphi_{/\beta} = 0$$

We call $\varphi_{/0} = \nu_0$ the frequency, and then we can prove the following statements : (*a*) ν_0 is constant along the light ray in every static field and in every static co-ordinate system; (*b*) ν_0 depends on the gravitational field at the point of emission (red shift)[1].

(2) *Based on Quantum Theory.* We can just as well define $\nu_0 = \nu$ in a natural way as the frequency. Let us define the inertial mass μ by:

$$\mu^2\left(g_{\alpha\beta}\frac{dx^\alpha}{dt}\frac{dx^\beta}{dt}\right) = m^2 = \text{constant}$$

$\mu\xi_{/0}{}^a$ is a vector with $\xi_{/0}{}^0 = 1$. Einstein's fundamental assumption for light rays and de Broglie's for the waves of matter is:

$$\mu\xi_{/0}{}^a = h\nu\xi_{/0}{}^a$$

where $\xi_{/0}{}^a$ is the velocity of light or that of the wave packet. It is natural to identify ν with the frequency, but to do so and also in order that (*a*) and (*b*) should be valid for ν, we must assume that h is a scalar density, and a quasi-Cartesian co-ordinate system has been chosen[2].

L. Infeld

University of Warsaw and
Polish Academy of Sciences.

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Measurements of Heats of Adsorption of Organic Vapours on Adsorbents of High Surface Area using a Continuous Flow Calorimeter

An apparatus for the measurement of heats of preferential sorption from liquid carriers by means of a continuous-flow system has already been described[1]. It was felt that this technique might also be applied to adsorption processes taking place from the vapour phase. A later model of the apparatus originally described was therefore modified to investigate the adsorption of organic vapours on to surfaces of high specific area.

Fig. 1 shows the calorimeter, in which the temperature-sensing elements comprise four thermistors, two reference thermistors situated in the body of the apparatus to ensure that they remain at constant temperature and a further pair of thermistors embedded in the surface of the adsorbent under investigation. Nitrogen is passed through the calorimeter at a rate of 50 ml./min., and a sample of gas, saturated with the vapour being used, is injected into the nitrogen stream by means of a long hypodermic needle. When the vapour reaches the adsorbent it is adsorbed and the rise in temperature measured and recorded by the thermistors, which are connected in a Wheatstone bridge network which feeds a 10-mV. potentiometric recorder via a 'Vibron' electrometer.

In order to obtain values for the heats of adsorption of the materials used the calorimeter was calibrated

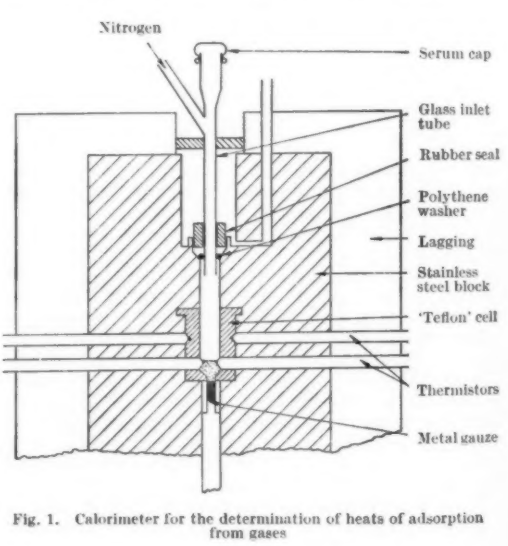

Fig. 1. Calorimeter for the determination of heats of adsorption from gases

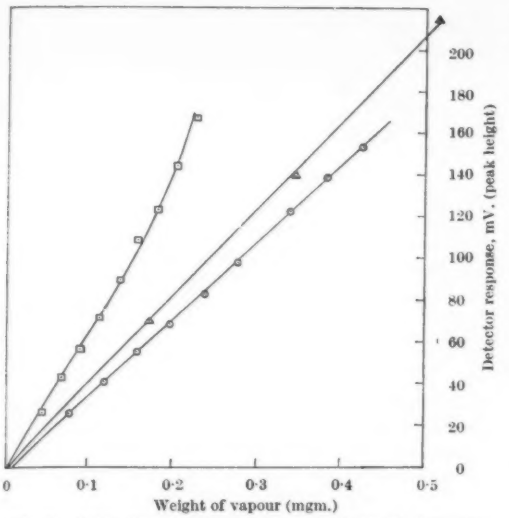

Fig. 2. Adsorption on silica gel. ○, *n*-heptane; △, benzene; □, isopropyl alcohol

by means of a coil of nichrome wire which was introduced into the surface of the adsorbent and through which known electric currents were passed. It was shown by this means that the response of the apparatus, in terms of peak height in mV. recorded, was linear with the quantity of heat dissipated in the adsorbent. An approximate value for the quantity of the materials contained in the samples of gas saturated with the vapour was obtained from the equation :

$$pV = \frac{w}{M} RT$$

where p is the vapour pressure of the sample at temperature $T°$ absolute, V the volume of gas in which the vapour is contained, w the weight of the vapour, M its molecular weight and R the gas constant. Vapour pressure values were obtained from the literature and from the weights thus obtained molar heats of adsorption were calculated.

Table 1. Adsorption on Silica Gel

Material	Temp. (°C.)	Response per 1·0 ml. vapour (mV.)	Response per μgm. (mV.)	Response per μmole (mV.)	Heat of adsorption (kcal./mole)
n-Heptane	22·0	71	0·33	33	12·1
Benzene	22·0	140	0·41	32	11·9
Toluene	22·0	45·5	0·38	35	12·9
Methanol	25·0	162	0·86	28	10·3
Ethanol	24·5	96	0·69	32	11·8
Isopropanol	21·0	71	0·63	38	14·0
1,2-Dichloroethane	23·5	102	0·29	28	10·3
1,2-Dibromoethane	22·0	22·5	0·18	35	12·9
Pyridine	22·0	37·5	0·63	50	18·4

Table 1 shows results obtained when 1·0-ml. samples of gas saturated with different organic vapours are adsorbed on to silica gel. It must be noted that the weights of the organic materials used are different in each case since a wide range of vapour pressures is covered; the surface coverages therefore differ widely. It was shown that for *n*-heptane the quantity of silica gel contained in the cell which takes part in the initial adsorption of the vapour was about 50 mgm., which corresponds to a surface coverage of approximately 0·1 μmol./m.², assuming that the sample is quantitatively adsorbed. The value of 12·1 kcal./mol. obtained for the adsorption of *n*-heptane is similar to values quoted in the literature for similar adsorbents[2].

When irreversible adsorption occurred it was found that on successive adsorptions of 1·0-ml. samples of vapour on to the same sample of silica gel the heat evolved decreased steadily. For example, the values obtained for benzene as shown in Table 1 are the means of a number of successive determinations. The initial response obtained for benzene adsorbed on to a fresh sample of gel was always higher than this, 155 mV. for 1·0 ml. sample of gas saturated with vapour at 22° C. This corresponds to an initial heat of adsorption of 12·9 kcal./mol., a value which might be expected in relation to 12·1 kcal./mol. for *n*-heptane[2]. For benzene and *n*-heptane the relationship between the quantity of vapour adsorbed, again assuming complete adsorption, and the response obtained, that is, the heat evolved, was found to be linear in the range of surface coverage investigated, up to about 0·2 μmol./m.² (Fig. 2).

Table 2. Adsorption on Activated Carbon

Material	Temp. (°C.)	Response per 1·0 ml. vapour (mV.)	Response per μgm. (mV.)	Response per μmole (mV.)	Heat of adsorption (kcal./mole)
n-Heptane	20·0	117	0·59	59	21·7
Benzene	22·5	235	0·65	51	18·8
Toluene	20·0	86	0·78	72	26·5
1,2-Dichloroethane	19·0	167	0·50	50	18·4
1,2-Dibromoethane	20·0	30	0·27	51	18·8
Pyridine	20·0	55	0·85	67	24·6

Activated carbon has also been used as the adsorbent and results obtained are shown in Table 2. Heats of adsorption on this material can be seen to be very much higher than in the case of silica gel. It has been reported[3] that heats of adsorption of this magnitude are obtained on adsorbents of this type when the surface coverage is low. This probably explains the very high value obtained for toluene in relation to that obtained for benzene, since the amount of toluene used was much less than that of benzene.

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G. H. Bell
A. J. Groszek

Petroleum Division,
BP Research Centre,
Sunbury-on-Thames,
Middlesex.

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An Experimental Apparatus for Contact Microradiography at 200–500 V.

Owing to the strong absorption of soft radiation, a sealed-off X-ray tube[1] is not suitable for contact microradiography. We have, therefore, used an X-ray tube without window.

The principle of the cathode/anode part is given in Fig. 1. A departure from conventional construction is made in order to comply with the requirements of small focus, high anode current and low accelerating

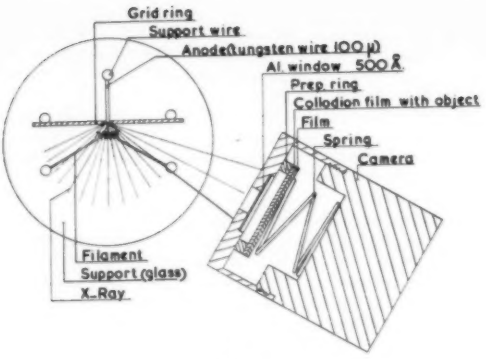

Fig. 1

voltage. The three components anode, grid, ring and filament are very simple to mount on a sintered glass bridge.

The cathode provides for the emission of the electrons which impinge mainly on the tip of the wire anode. The ring ensures that no electrons are deflected beyond the edge of the anode by either electrostatic or mechanical means; this is in order to prevent X-rays from being produced there also. A voltage of from +10 to +20 V. at the ring is sufficient. The diameter of the hole is 0·7 mm. Overload of the anode need not be feared, since the load is of the order of 0·2 W. continuous.

As can be seen from the diagram, the camera itself can be mounted in a position as favourable as possible with respect to the anode. The aperture of the camera has to be made light-proof by means of an aluminium membrane[2]. In our tests we used a shadow-casting apparatus. In principle, any evacuated vessel is suitable, provided it has three connecting wires: two for the cathode and one for the ring.

The electrical part consists of a filament-current transformer for the cathode, and a source of direct voltage for the ring and the anode, of 10 and 200–500 V. respectively.

The advantage of the microradiographic method is that the soft radiation can provide information which may be correlated with the mass and chemical composition of the microscopic objects. The resolving power is about the same as that of light microscopes[2–4].

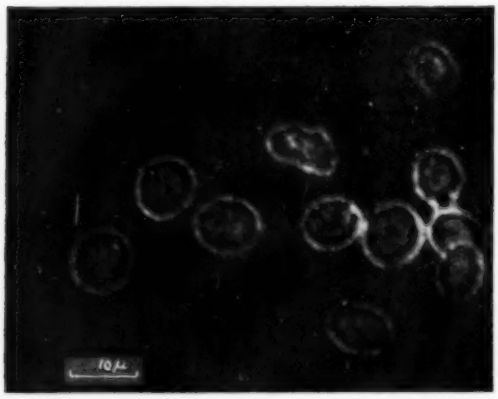

Fig. 2

The preparation techniques employed are those of Greulich and Engström[2]. An example of the results obtained is given in Fig. 2, which shows a contact microradiograph of erythrocytes (350 V., 0·4 m.amp., exposure time 5 min. Eastman Kodak Spectroscopic Emulsion 649–0. Distance from X-ray source and emulsion, 6 mm.).

A. Recourt
G. H. F. de Vries

Physical Technical X-Ray Laboratory,
N.V. Philips' Gloeilampenfabrieken,
Eindhoven.

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Irradiated Lithium Fluoride as an Optical Filter in the Far Ultra-Violet

Photochemical studies of aqueous solutions of organic compounds are being carried out at this Laboratory using the 1849 Å. radiation emitted by a low-pressure mercury resonance lamp. It has been found necessary to eliminate the contribution of the intense 2537 Å. line emitted by such a source. This has been done by using a filter of irradiated lithium fluoride. The idea of using this material as the filter for mercury resonance radiation was suggested to us by P. Yuster of this Laboratory. The absorption spectrum of X-irradiated lithium fluoride shows a sharp band with a maximum at 2420 Å. due to the formation of *F*-centres in the crystal[1]. Even with complete absorption at this wave-length, the transmission of the irradiated crystal remains high at 1849 Å.

Polished disks of lithium fluoride, 40 mm. in diameter and 0·8 mm. in depth, were prepared from a crystal obtained from the Harshaw Chemical Co. Irradiations were carried out with a high-intensity cobalt-60 source of γ-radiation at several dose-rates. Spectral measurements were made with an accurately calibrated Cary model 14 recording spectrophotometer.

The increase in absorption at 1849 Å. and 2537 Å. with radiation dose is shown in Fig. 1, where dose is plotted against T/T_0, the ratio of transmissions after and before irradiation. The absorption spectra of the irradiated filters between 1849 Å. and 4700 Å. are similar to those recently reported by Kato *et al.*[2]. The decrease in transmission at 1849 Å. is due to the tail of the *F*-band.

After irradiation the crystals were exposed to a high-intensity mercury resonance lamp for various lengths of time, and it was found that the rate of bleaching of the *F*-centres was low enough to allow the crystals to be used as filters. The experimental results for a typical filter are shown in Table 1.

Table 1. Transmission of Lithium Fluoride Filter at 1849 Å. and 2537 Å. after Irradiation and Bleaching

Treatment		Percentage of transmission at 1849 Å.	Percentage of transmission at 2537 Å.
Cobalt-60 γ-irradiation	Bleaching* (2537 Å.)		
None	None	70	85
1·7 Mrad	None	40	< 0·1
1·7 Mrad	2×10^{20} quanta	47	< 0·1
1·7 Mrad	4×10^{20} quanta	49	~ 0·1
1·7 Mrad	6×10^{20} quanta	50	~ 1

* Lamp intensity, I (2537 Å.) ~ 10^{17} quanta sec.$^{-1}$.

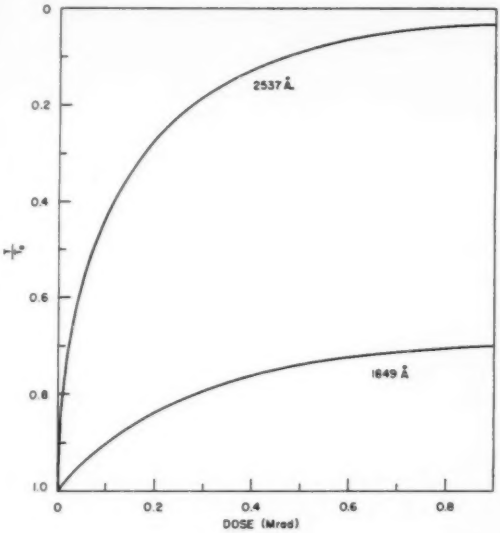

Fig. 1

This work was performed under the auspices of the United States Atomic Energy Commission.

J. L. Weeks
S. Gordon
G. M. A. C. Meaburn

Argonne National Laboratory,
Argonne, Illinois.

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CRYSTALLOGRAPHY

X-ray Diffraction Spikes of Diamond

The anomalous X-ray reflexions of 'diffraction spikes' from type I diamonds have been studied by a number of authors[1–5] since they were discovered by Raman and Nilakantan[1].

Frank[4] explained the diffraction spikes, on the basis of the detailed data about the intensity of spikes by Hoerni and Wooster[3], as being due to segregated (100) planes of impurity atoms. He considered one central plane of atoms with spacing $(1+\delta)$ units of length to its neighbour planes, all interplanar spacings thereafter being 1. Thus calculating one-dimensionally, the amplitude of the spikes belonging to each reciprocal point h will be ultimately proportional to $\sin h\theta$ ($\theta = 2\pi\delta$). Frank evaluated $2\theta = 180° + 2\varphi + n \times 360°$, $2\varphi = 52°\ 17'$ or $67°\ 9'$ (n; integer) from $\pm\sqrt{(5/100)} = \sin 30/\sin\theta$, using the observed value of Hoerni and Wooster in $h = 1,3$. Also, he calculated with this θ the intensity in $h = 1$–5 with the results as shown in Table 1. The amplitude in the case of $h = 0$ was given separately by the equation:

$$A = B - 1 - 2\delta \qquad (1)$$

where B is the scattering weight of the impurity planes when the weight of carbon planes is assumed as 1. Considering that this observed value becomes almost zero, as well as the valency condition and size effect by using the result of the above calculation, the impurity atoms were considered to be silicon.

Table 1. Intensity of Diffraction Spikes of Diamond

h	Hoerni and Wooster observed value	Frank calculated value		Yoneda calculated value
		$2\varphi = 52°\ 17'$	$2\varphi = 67°\ 9'$	
0	< 5	0	0	0
1	100	100	100	58
2	75	77	122	38
3	5	5	5	7
4	30	116	70	68
5	absent	53	138	17
6	—	19	20	24

Caticha-Ellis and Cochran[5], who evaluated the impurity concentration, by their detailed calculation and experiment have criticized Frank's calculation as giving a concentration of silicon more than 80 times larger than that actually present.

I have used Frank's method[4] of calculation in its original form. It is, however, not effective, since the value 5 is too small in intensity and large in relative error to determine θ only from 100/5 without recourse to other available data. Accordingly, here θ and K have been determined by using all measured values and the method of least squares, putting $K \sin^2 h\theta = L_h$ ($h = 1$–5). (L_h is the intensity of spikes, K is a constant.) As the result, the most probable value obtained was $2\theta = 2\varphi + n \times 360°$, $2\varphi = \pm(132° \pm 3°)$, $K = 69 \pm 19$, which is quite different from Frank's calculation in spite of the same data being used. The result of evaluating the intensity of spikes by using this value as Frank did is shown in Table 1. This result seems to be a little nearer than Frank's to the observed value as a whole. The most probable value of θ, however, is more reliable than these calculated values of intensity. Frank has calculated the Si—C bond-length as 1·95 Å. or 1·935 Å., but the calculation of the bond-length corresponding to this by using my most probable value gives 1·45 Å. or 1·64 Å.

Kaiser and Bond[6] proved that many type I diamonds contain about 0·2 per cent of nitrogen as impurity. They also confirmed that this nitrogen is characteristic of type I diamonds.

Smith *et al.*[7] have found that there is a C—N bond in about half the diamond samples by electron-spin resonance experiments. According to them, the result of electron-spin resonance can be explained by assuming the C—N bond-length of one of the four bonds as having increased by several per cent, since the nitrogen donor electron has the tendency to take an antibonding orbit due to the Jahn–Teller effect. I believe, therefore, that 1·64 Å. should be adopted, which is 6·5 per cent larger than the 1·54 Å. for the C—C bond-length and nearly corresponds with the results obtained by Smith *et al.*

Also, assuming the impurity to be nitrogen, the spike amplitude of $h = 0$ is as weak as 0·2 from equation (1), which approaches Hoerni and Wooster's observed value[3], even if it is not zero as is calculated by Frank[4].

Evaluating the concentration of nitrogen by δ from my calculation and using the result of experiment and the equation of Caticha-Ellis and Cochran[5], about 0·8 per cent is obtained. Though wide differences in the nitrogen content of various samples have been pointed out by many authors and the nitrogen content of the samples used by Caticha-Ellis is not known, this result seems to be too large compared with the result of analysis by Kaiser and Bond[6].

I wish to thank Prof. T. Futagami for valuable discussions.

Y. Yoneda

Department of Applied Physics,
Faculty of Engineering,
Kyushu University,
Fukuoka, Japan.

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The quantity 2φ defined by Yoneda for his own calculations differs from my quantity 2φ, the definition of which he quotes earlier, by 180°. Consequently his result 132° ± 3° becomes, in my notation, 48° ± 3°, which is not very different from my 52°. Whether it is superior is very debatable. Yoneda's value increases the discrepancy from the observed spike intensities (relative to that at first order) in the second, third, and fourth orders, without gaining any satisfactory agreement at fifth order, for which he still obtains a stronger spike than at third order, instead of an absent one. I supposed another factor to be present, giving a general falling off of intensity at higher order. Yoneda's calculation does not dispose of the evidence for such a factor, but if such a factor were present, his least squares analysis is less appropriate than my method of calculation, the essential point of which is to give great weight to the location of a minimum (a zero, according to the theory) near the third order. The most remarkable result is the smallness of the change which his method of calculation brings about.

The bond-lengths 1·45 Å. or 1·64 Å. which Yoneda calculates are members of a series of bond-lengths (between carbon atoms and an assumed monolayer of impurity atoms) which would all give the same diffraction spikes except at zero order, namely :

1·399, 1·453, 1·640, 1·719, 1·955, 2·046 ... ...

for Yoneda's value ($2\varphi = 48°$) or

1·395, 1·457, 1·633, 1·723, 1·950, 2·053 ... ...

for my value ($2\varphi = 52°$)

while my less preferred value ($2\varphi = 67°$) would give bond-lengths about another 0·012 Å. alternately smaller and greater than the latter list. The associated numbers for the impurity to give minimal spike intensity at zero order are in all three cases:

2, 4, 8, 10, 14, 16 ...

It is clear on this basis that if the impurity were to be identified as nitrogen ($Z = 7$), the third member of this series should be selected, and, as Yoneda remarks, this gives a credible bond-length, namely, 1·640 Å. according to Yoneda, or 1·633 Å. or 1·620 Å. (with preference for the former) according to me.

The basis for Yoneda's revision of Caticha-Ellis and Cochran's estimate of atomic concentration of impurity from 8×10^{-4} to 8×10^{-3} is not apparent: no acceptable change in δ should alter the result by so much. On the contrary, Caticha-Ellis and Cochran's estimate must be essentially independent of the nature of the defect, within its acceptable range, so that Kaiser and Bond's nitrogen contents are adequate to produce the diffraction spikes.

While this gives a credible interpretation of the 'spike-producing' defects, it must be borne in mind that the multiplicity of alternative magnitudes of displacement giving similar diffraction results makes the diffraction evidence alone incapable of discriminating against other possible models (such as one with nitrogen atoms in two layers). The matter can scarcely be settled before we have X-ray diffraction, infra-red absorption, and spin-resonance measurements all on the same diamond.

F. C. Frank

H. H. Wills Physics Laboratory,
Royal Fort,
Bristol 8.

BIOPHYSICS

Proposed Use of Magnetic Fields in Electron Microscopic Radioautography

The use of strong magnetic fields has been proposed as a possible way of increasing resolution in radioautography[1]. It was reasoned that a sufficiently strong magnetic field would bend the path of emitted β-particles into a circle of arbitrarily small radius, thereby restricting the resulting radioautographic grains to a small zone around the source. In recent reports[2,3], Harford and Hamlin, using the same argument, attempted to improve the resolution of electron microscopic radioautographs by placing the specimens in a 10-kilogauss magnetic field during exposure. They could not demonstrate such an improvement; but they attributed a considerable increase in the number of exposed grains to the effect of the field on the β-particles from tritium.

A simple calculation shows, however, that magnetic fields at present available can have little influence on the path in emulsion of low-energy β-particles. Tritium emits electrons of approximately 18-keV. maximum energy and 5·5-keV. average energy. Let us take the case of a 10-keV. electron (in the high-energy side of the spectrum, but with still a fairly high probability of occurrence). For such an electron moving perpendicularly to the lines of force of a uniform magnetic field the product $HR = 3{\cdot}386 \times 10^2$ gauss cm. (ref. 4) (where H = magnetic field intensity and R = radius of curvature). For a commonly available field of 10^4 gauss $R \simeq 3{\cdot}4 \times 10^{-2}$ cm. = 340μ. The maximum range of a 10-keV. electron in material of density 1 is calculated to be of the order of 2·5μ (ref. 5). In emulsion it is probably about one half this value. On such a short path a curvature of radius 340μ would have no detectable effect. As the electron slows down, the radius of curvature will decrease, but, even for a 1-keV. electron with a calculated range in water of 0·053μ, the radius of curvature would still be about 100μ.

We may ask what fields would be needed to have a useful influence. A resolution of the order of 0·2–0·3μ can be theoretically expected (unpublished results), and experimentally demonstrated[6], when a monolayer of a fine-grained emulsion is used in electron microscopic radioautography[7]. We can ask, therefore, that the field be of sufficient strength to give a 10-keV. electron a curvature of 0·1μ radius. Taking $HR \simeq 3{\cdot}4 \times 10^2$ gauss. cm., we get, under these conditions, $H = 3{\cdot}4 \times 10^7$ gauss. This is approximately one hundred times stronger than any permanent field yet achieved. Since the change in electron

velocity in going through matter is small we have used in these calculations the velocity *in vacuo*.

It might be added that, even with stronger magnetic fields, an increase in grain counts could not easily be explained on the basis of an altered path of the electrons. Assuming the field to be perpendicular to the horizontal plane of the section the vertical component of velocity of emitted electrons would be unaffected, while the horizontal component would be changed only in direction but not in magnitude. The total length of the path in emulsion would, therefore, remain the same, since the time spent by the electron in the emulsion depends only on the vertical component.

It does not seem that magnetic fields can be used effectively to modify the characteristics of radioautographs with tritium. A similar situation prevails in the cases of carbon-14 and sulphur-35. Only in the case of phosphorus-32 do the radii of curvature become of the same order as the ranges. But since in that case the entire path of the particle appears as a track there seems to be no great advantage in modifying its shape. It is therefore likely that the increase in grain counts observed by Harford and Hamlin is due to causes other than the effect of the magnetic field on the path of the β-particles.

LUCIEN G. CARO

The Rockefeller Institute,
New York 21.

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GEOLOGY

Uranium in British Surface and Underground Waters

RECENT interest in the alpha-activity of potable water[1] has prompted the following summary of the results of analyses for uranium in surface and underground waters, obtained during an assessment of uranium mineral resources in Great Britain (Table 1). Most of the samples were collected by the Atomic Energy Division of the Geological Survey of Great Britain and the uranium content determined by the National Chemical Laboratory, the Atomic Energy Research Establishment, Harwell, and the Atomic Energy Division. The analytical techniques were: the colorimetric method developed by Smith and Chandler[2], with later modifications by Hunt[3]; the simple fluorimetric method of Milner and Barnett[4]; and the fluorimetric method normally used in the laboratory.

Average background values based on 678 samples taken from surface waters in south-west England, north England and west Scotland are 1γ/l. or less (1γ/l. equals 10^{-6} gm./litre). Higher values in streams emerging at the limestone/shale contact in the Castleton area of Derbyshire can be related to leaching of uraniferous phosphate in the limestones[5] and to high bicarbonate ion concentration, the latter being well known to influence the solution of uranium in natural waters. Conversely, the low bicarbonate ion concentration probably accounts for the occurrence of small quantities of uranium in the Dalradian rocks of Knapdale[6] not being reflected in the analyses of water samples from that area. The value of 50γ/l. in water from the Goon Vean china clay leat is due chiefly to uranium from the artificially introduced particulate matter (mica and clay minerals) suspended in the water[2]. Filtered water from the same source contains less than 1γ/l.

As is to be expected, some underground waters contain higher concentrations of uranium than surface waters. The highest recorded uranium concentration (30–50γ/l.) is in water from a shaft at the disused South Terras uranium mine. Uranium concentration in spa waters is consistent with their origin, composition, and extended contact with crustal rocks.

Lack of consistency between the results of analyses undertaken at different times on Cornish mine waters is considered to be due to the effect of differing weather conditions immediately preceding the collection of samples, since checks have shown that comparable results are obtained with the different analytical techniques used. Measurements of the uranium content of waters in Great Britain and abroad have indicated that the amount of dissolved uranium reaches a maximum when rain follows a dry spell during which normal weathering processes in the zone of oxidation have made available abnormal quantities of uranium in a readily soluble form. The range of values recorded over a period of several weeks at St. Anne's Well, Buxton (from four samples), may also be attributable to varying ground-water circulation.

Table 1. URANIUM CONCENTRATIONS IN SOME SURFACE AND UNDERGROUND WATERS

District	Locality	Year	Uranium, γ/l.	Type of water	Technique
South-West England	St. Ives' Water Supply	1956	1·2	mine	fluorimetric and colorimetric
		1958	8·6		
	Tretheague	1958	1·0	radioactive spring	colorimetric
	Pengreep	1958	1·0–5·0	mine	colorimetric
	Wheal Speed	1958	7·0–8·0	mine	fluorimetric and colorimetric
	South Terras Shaft	1958	30–50	mine	fluorimetric and colorimetric
	Goon Vean	1958	50	china clay leat (unfiltered water)	fluorimetric and colorimetric
	Wheal James	1956	10	mine	fluorimetric
	Adit	1958	2	mine	colorimetric
	Wheal Bray	1956	10	mine	fluorimetric
	Winze	1958	2	mine	colorimetric
West England	Cheltenham Spa	1958	2·3	Spa	fluorimetric
Derbyshire	St. Anne's Well, Buxton	1958	2·5–6·0	Spa	fluorimetric and colorimetric
	Riber Mine, Matlock (western end of 220-ft. level)	1958	4	mine	fluorimetric and colorimetric
	Russel Well, Castleton	1958	2	spring	fluorimetric and colorimetric
	Spring, Grid Ref. 154837	1958	13–15	spring	fluorimetric and colorimetric
Cheshire and Shropshire	White Grit Mine	1959	2·6	mine	fluorimetric
	Eardiston Copper Mine	1959	2·3	mine	fluorimetric
Argyllshire, Knapdale	Achaglachgach	1959	1·7*	stream	fluorimetric
	Kilberry	1959	1·5*	stream	fluorimetric

* The highest uranium concentration found in the area.

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J. D. Peacock

Atomic Energy Division,
Geological Survey of Great Britain,
Young Street, Kensington,
London, W.8.

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Continuation of the Great Glen Fault Beyond the Moray Firth

During 1944–45 I was stationed in Fair Isle, Shetland, during which time I was able to make a number of general observations on the geology of the island which tend to support Dr. Flinn's hypothesis[1] that the Great Glen Fault continues northwards, past the west coast of Fair Isle, to Shetland, where it is called the Walls Boundary Fault. Because of the inaccessibility of the Isle and its important position relative to the suggested course of the fault, it is worth while recording the observations; but it must be emphasized that they are recollections, supported by only a few photographs, specimens and notebook data.

Fair Isle is composed mainly of a barren series of shales, sandstones and conglomerate of Old Red Sandstone type, which the Geological Survey doubtfully identify as Lower Old Red Sandstone[2]. A detailed search for fish-beds was made but without success. The beds dip approximately to the south-east[3] with dips ranging from about 60° on the east side of the island (Fig. 1) to very nearly vertical on the west. On the east and north sides the rocks are unaltered, but towards the south-west corner of the island the shales and sandstones appear to pass into phyllites and low-grade schists containing quartz lenticles. Several of the pebble beaches in this part of the island were made up, almost entirely, of a dark, fine-grained, but porphyritic rock containing phenocrysts of plagioclase, indicating that igneous rocks were probably *in situ* just off the coast, if not in the inaccessible cliffs of Malcolm's Head at the south-west of Fair Isle. Farther north on the west coast, one or more dolerite dykes cut the cliff in an east–west direction.

When compared with Orkney, to the south, and Shetland, to the north, the most surprising feature is the attitude of the rocks, for in Orkney and Shetland the Old Red Sandstone has low dips of the order of 40° or less in the limbs of the gently rolling anticlines and synclines[4]. The fact that the dips become increasingly steep and approach the vertical towards the west coast of Fair Isle would accord with the hypothesis that a large disturbance lies to the west.

A further point is that erosion on the east side of the island reflects the lithology and structure of the rocks, soft bands being removed and more resistant beds standing up as headlands, the whole relief being aligned along the north-east–south-west strike. On the west side, however, erosion has been along joints and minor faults, and erosion features are parallel to the direction of dip. This tends to mask the fact that the general trend of the west coast does not coincide with the strike of the rocks but is about parallel to the trend of the Great Glen Fault extension as suggested by Dr. Flinn.

Fig. 1. Old Red Sandstone dipping to the south-east at a high angle on the east coast of Fair Isle, Shetland

Lastly, there is no large-scale shattering of the rocks in Fair Isle. This is also to be expected if the Great Glen Fault occupies the position suggested by Dr. Flinn, for in the Seli Voe area of Shetland there is shattering for distances of more than a mile from the fault, while Fair Isle lies about four miles east of the supposed position of the fault. The fault zone and the zone of shattering have been removed, as they are being rapidly removed at the present day at Seli Voe, Aith Voe and Brae.

Thus, the increase of the dip towards the west coast of Fair Isle, the trend of the west coast-line and the absence of large-scale shattering are three pieces of geological evidence which accord with Dr. Flinn's hypothesis.

Robert M. Appleby

Department of Geology,
University College,
Cardiff.

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Lung-books in the Devonian Palæocharinidae (Arachnida)

The typical breathing organs of most of the larger recent Arachnids are lung-books. These are thought to have arisen from the segmentally arranged gills of an aquatic ancestor. The presence of lung-books in fossil Arachnids is thus of importance in any phylogenetic studies on the group. Petrunkevitch[1–3] has reviewed the known Palæozoic and Mesozoic fossils and shown the presence of paired structures on the proximal opisthosomal sternites of a few forms, particularly among the Carboniferous Anthracomartida

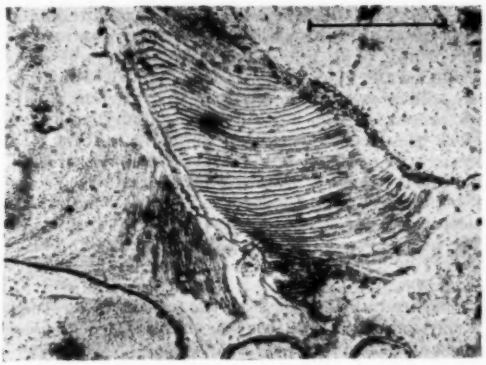

Fig. 1. Detailed structure of a single lung-book (scale 0·5 mm.)

and Trigonotarbida. These he has reasonably interpreted as representing the stigmata of lung-books. However, there seem to be no records of definite lung-book structure in any fossil.

The fine structure of lung-books may only be expected in fossils from deposits formed under exceptionally favourable conditions of preservation. Such a deposit is the Rhynie Chert (Scottish Middle Old Red Sandstone). Remarkably well preserved Arachnids attributed to the family Palæocharinidae, recently placed in the order Trigonotarbida by Petrunkevitch[1,2], have been described by Hirst[4], who was able to give full descriptions of external features but made no mention of lung-books or their stigmata.

During a re-investigation of the plants of the chert carried out by one of us (A. G. L.), numbers of these Arachnids have been encountered together with the plants among which they lie. Thin serial sections have been made by means of the palæobotanical peel method, after etching with hydrofluoric acid. Some of these sections show clear evidence of lung-book structure (Figs. 1 and 2), resembling very closely the comparable organs of modern spiders. Longitudinal sections show the presence of two pairs apparently opening by small stigmata on the sternites of the first and second opisthosomal segments (Fig. 2). With this and further sectioned material it should be possible to make detailed reconstructions and identifications.

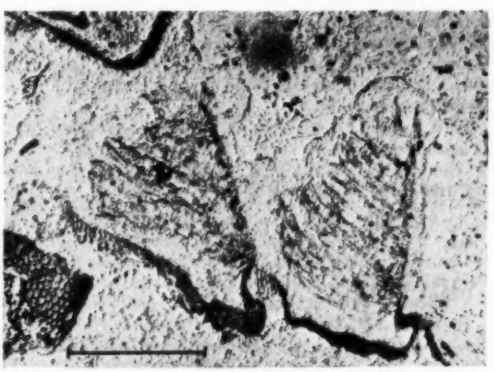

Fig. 2. Longitudinal section of successive lung-books and stigmata (scale, 0·1 mm.)

The Palæocharinidae have been recorded only from the Rhynie Chert and are of particular interest since they are among the earliest terrestrial Arachnida known with any certainty, and because they show a number of important similarities to the Araneida. To the latter can now be added the presence of two pairs of lung-books.

M. F. Claridge

Department of Zoology,

A. G. Lyon

Department of Botany,
University College,
Cardiff.

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CHEMISTRY

Complex Compounds of Zero-valent Platinum

Recently, Chopoorian, Lewis and Nyholm[1] have indicated that the triphenylphosphine complexes of platinum(0) described by Malatesta and Cariello[2] are hydrides $[PtH_2(PPh_3)_n]$ (n = 2, 3 or 4), and question the existence of any platinum(0) complexes. Some years ago we prepared a compound $[Pt(Ph_2PCH_2CH_2PPh_2)_2]$. We have now examined it very carefully and conclude that it is indeed a complex of zero-valent platinum.

It is precipitated during the vigorous reaction which ensues on the addition of concentrated aqueous sodium borohydride solution to a hot ethanol/water (8 per cent) solution of $[Pt(Ph_2PCH_2CH_2PPh_2)_2]Cl_2$, and crystallizes from benzene/methanol in golden yellow plates, m.p. 252–255°, in an evacuated tube. It is a non-electrolyte of very low dipole moment, and oxidized only very slowly by air. The infra-red and nuclear magnetic resonance spectra show no sign of Pt—H bonds or hydridic hydrogen. On oxidation in benzene solution with an excess of iodine (3 mol.) and extraction with water no acidity is developed, as it would were the complex a hydrido-complex.

With Dr. H. R. Watson's assistance we have also obtained a purely aliphatic analogue $[Pt(Me_2PCH_2CH_2PMe_2)_2]$ by the reduction of the corresponding dipositive cation with sodium naphthalenide (NaC_8H_{10}) in tetrahydrofuran[3]. It is a colourless substance highly sensitive to air.

It is presumed that both these d^{10} complexes will have a tetrahedral configuration, corresponding to that of nickel carbonyl.

We thank Dr. N. Sheppard for examination of the nuclear magnetic resonance spectra.

J. Chatt
G. A. Rowe

Imperial Chemical Industries, Ltd.,
Heavy Organic Chemicals Division,
Akers Research Laboratories,
The Frythe,
Welwyn, Herts.

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Reduction of Zirconium and Hafnium Oxides

Many attempts have been made to reduce zirconium dioxide with hydrogen. At temperatures up to 1,500° C. no positive results have been reported[1–3]. Newbury and Pring[4] reported that at 2,500° C. and a hydrogen pressure of 150 atm. no reduction took place. Mott[5] stated that in an electric arc at 6,000° C. and at a hydrogen pressure of 22 atm. reduction was difficult but that sometimes a small amount of "yellow Zr_2O_3" was produced. In the reduction of ZrO_2 with lithium or calcium hydride it has been suggested that the active reducing agent is atomic hydrogen. Simonenko[6], using a d.c. arc between tungsten electrodes, in a hydrogen atmosphere, claimed to have produced metallic zirconium from ZrO_2 placed in the cathode cavity. He believed that the ions of molecular and atomic hydrogen were responsible for the reduction, their effectiveness being increased by the negative potential.

Atomic hydrogen produced by passing hydrogen at low pressure through an electromagnetic field of 29 Mc./s. or lower (Blackwood and McTaggart[7]) will reduce many oxides but not ZrO_2. However, it was found that if the frequency of the exciting field was increased to 2,500 Mc./s. a surface layer was formed which was black or golden. This material had a specific resistivity of the order of 1 ohm-cm. although measurements were difficult for obvious reasons. It has not been possible so far to determine the Zr:O ratio, but X-ray diffraction indicated that no change of phase occurred, the pattern remaining that of the dioxide. During treatments the temperature of the samples varied from about 200° C. to 1,200° C. depending on the power supplied (50–400 watts input to the magnetron).

Hafnium oxide, when treated in the same way, also became black and conducting. Thorium oxide remained unchanged. It would appear that a reduction took place in the case of ZrO_2 and HfO_2 similar to that occurring when TiO_2 is reduced in hydrogen at red heat. In the 2,500 Mc./s. field the energy normally possessed by hydrogen atoms is presumably increased by further excitation and ionization, as visualized by Simonenko, to a value which permits reduction of the above oxides to proceed.

F. K. McTaggart

Commonwealth Scientific and
Industrial Research Organization,
Division of Mineral Chemistry,
Fishermen's Bend,
Victoria.

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Irradiation Decomposition of Carbon Dioxide

During investigation of the reaction between carbon dioxide and graphite induced by radiation, we have examined the decomposition by irradiation of carbon dioxide in the absence of graphite. These irradiations have been carried out in transparent silica vessels into which the required amounts of carbon dioxide are condensed before sealing off. Particular care was taken to exclude organic contaminants by cleaning the silica vessels, wool and rods in hot chromic acid, followed by three washings in hot distilled water and firing at 1,000° C. for 2 hr. in an atmosphere enriched with oxygen. Carbon dioxide was prepared from 'Cardice', distilling three times before use, with rejection of first and last fractions. Mercury vapour was eliminated by the use of a trap at −78° C. placed immediately before the vessels to be filled. Analysis of the gas after irradiation was carried out by a gas chromatographic method[1]. The samples received a thermal neutron dose of $2{\cdot}3 \times 10^{18}$ n.cm.$^{-2}$ in the core of the Harwell reactor *Bepo*. From the integrated dose of thermal neutrons as measured by cobalt wires, the energy dose received by the carbon dioxide was calculated using the calorimetric data of Anderson and Waite[2].

Four general conclusions may be drawn from the results :

(1) The decomposition by irradiation of carbon dioxide in the absence of an oxygen acceptor is extremely small. (2) An increase in the surface area of the silica as effected by the introduction of silica wool into the vessels does not increase the efficiency of carbon monoxide production (the G_{CO} value). The higher G_{CO} values obtained using vessels packed with silica rods are inexplicable at present. (3) An increase in the carbon dioxide pressure up to 9·5 atmospheres at the operating temperature of 100° C. does not cause a major change in G_{CO}. (4) There is always a deficiency of oxygen compared with the carbon monoxide formed.

Table 1. Radiation-induced Decomposition of Carbon Dioxide in Empty and Packed Silica Vessels

Empty vessels, surface area ~ 50 cm.² filling pressure (at 0° C.) mm. mercury	G_{CO}	Rod-packed vessels, surface area ~ 200 cm.² filling pressure (at 0° C.) mm. mercury	G_{CO}	Wool-packed vessels surface area ~ 1,200 cm.² filling presure (at 0° C.) mm. mercury	G_{CO}
42	< 0·03	66	0·21	42	< 0·007
81	< 0·004	142	0·04	169	< 0·002
158	< 0·002	287	< 0·009	307	< 0·001
343	< 0·001	596	< 0·015	671	< 0·001
629	< 0·001				
807	< 0·002				
1,761	< 0·002				
2,130	< 0·001				
3,237	< 0·001				
3,967	< 0·001				
5,327	< 0·001				

The first of these conclusions is in agreement with the earlier work of Harteck and Dondes[3], but the second and third conclusions are in direct contradiction to their work. The formation of ozone in the radiolysis of carbon dioxide as postulated by Harteck and Dondes would explain the oxygen deficiency in the present work. This would also agree with the recent work of Mahan[4] on the photochemical decomposition of carbon dioxide.

It is hoped to publish more details of this work at a later date. The support and irradiation facilities of the United Kingdom Atomic Energy Authority are gratefully acknowledged.

T. B. Copestake
N. S. Corney

General Electric Co., Ltd.,
Central Research Laboratories,
Hirst Research Centre,
Wembley, Middlesex.

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Mechanism of Synthesis of Adenine from Hydrogen Cyanide under Possible Primitive Earth Conditions

It has recently been shown that adenine and the purine precursors 4-aminoimidazole-5-carboxamidine and formamidine are formed spontaneously from hydrogen cyanide in water–ammonia systems under conditions assumed to have existed on the primitive Earth[1].

I wish to propose now a mechanism for the formation of adenine from hydrogen cyanide under the conditions of the above experiments which is in line with previous observations[2]. Adenine formation from hydrogen cyanide is thought to be initiated by base catalysis and to require the participation of 4-aminoimidazole-5-carboxamidine and formamidine as the key intermediates. The different steps involved in this synthesis are summarized in Fig. 1 and briefly described below. Direct experimental evidence for the last and most important step in this synthesis is also given below.

Reaction 1. In the presence of ammonium hydroxide, cyanide ion is formed which condenses with hydrogen cyanide to form iminoacetonitrile. Indirect evidence for the formation of iminoacetonitrile as the primary product of the base-catalysed hydrogen cyanide polycondensation has been obtained from kinetic studies on the formation of polymeric hydrogen cyanide[3].

Reaction 2. As soon as iminoacetonitrile is formed cyanide ion condenses with this compound to form aminomalonodinitrile. The transformation of hydrogen cyanide into a hydrogen cyanide trimer, presumed to be aminomalonodinitrile, although not completely elucidated, was claimed a long time ago by several investigators[4], and this trimer of hydrogen cyanide, of its isomer α,β-diiminopropionitrile, has also been considered as an intermediate in the formation of tetrameric hydrogen cyanide[5].

Reactions 3 *and* 4. Hydrogen cyanide as well as aminomalonodinitrile are ammonolysed into the corresponding amidines, formamidine and aminomalondiamidine. These reactions deserve no special comment, since the ammonolysis of nitriles is well known, and is in fact one of the main methods used in the preparation of amidines.

Reaction 5. Aminomalondiamidine condenses with formamidine to yield 4-aminoimidazole-5-carboxamidine. Indirect evidence for this reaction can be found in the very similar syntheses of 4-amino-5-substituted imidazoles carried out by other workers[6]. In the experiments carried out in my laboratory the appearance of 4-aminoimidazole-5-carboxamidine as well as formamidine among the reaction products was observed very early during the course of the hydrogen cyanide condensation reaction. In addition to 4-aminoimidazole-5-carboxamidine, 4-aminoimidazole-5-carboxamide was also isolated from the reaction mixture. The latter compound was observed to be formed by hydrolysis of the former, as was to be expected from the strongly basic conditions of the reaction mixture.

Reaction 6. In the final step, 4-aminoimidazole-5-carboxamidine condenses with another molar equivalent of formamidine to yield adenine. In support of this reaction, preliminary kinetic studies on the formation of formamidine and 4-aminoimidazole-5-carboxamidine have shown that these compounds make their appearance and disappearance in the reaction mixture as if they were intermediates of adenine. The cyclization of 4-aminoimidazole-5-carboxamidine with formamidine into adenine was thus considered the key step in the elucidation of the mechanism of adenine synthesis. For this reason, and because the reactants were easily available, an independent experimental demonstration of this reaction was undertaken.

This cyclization reaction was studied under different conditions of *p*H and carried out as follows: About 50 mgm. of 4-aminoimidazole-5-carboxamidine and 90 mgm. of formamidine hydrochloride were dissolved in 1 ml. of distilled water. Exactly 0·2 ml. aliquots of this solution were mixed with an equal volume of either water, 1 *M* disodium hydrogen phosphate, or 7 *N* ammonium hydroxide. The *p*H of the resulting reaction mixtures was approximately 1, 7 and 12 respectively. These three mixtures were heated in closed vessels at a temperature of 130° C. for 12 hr. At the end of the reaction time, a very small amount of residue was separated by centrifugation from each reaction product and each of the supernatants was analysed by paper chromatography and ultra-violet

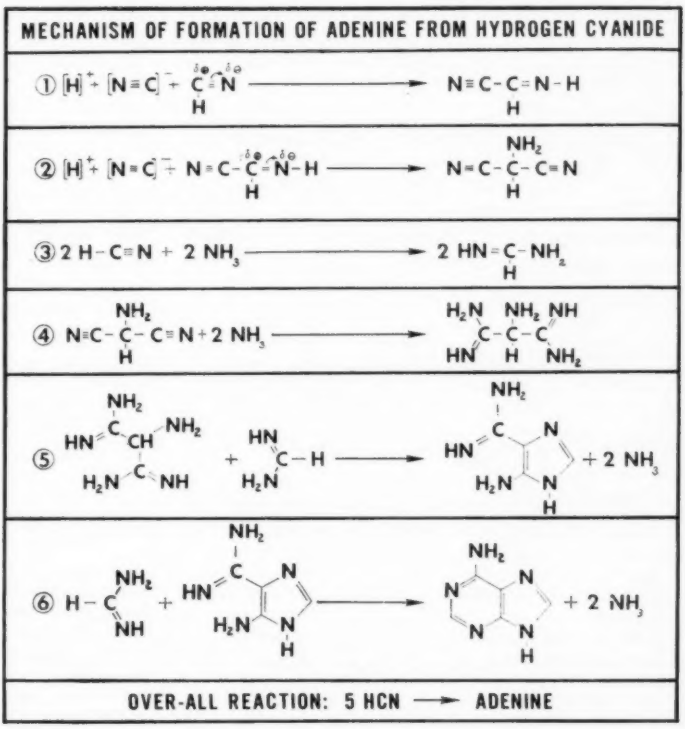

Fig. 1

spectrophotometry as described previously[1]. In all cases adenine was formed in high yield. Some unreacted 4-aminoimidazole-5-carboxamidine was observed in the acid and neutral reaction mixtures, but none in the basic reaction mixture. The experiment has been repeated recently at lower temperatures, and similar results have been obtained.

Although alternative mechanisms cannot yet be ruled out, the evidence obtained in my laboratory as well as recent evidence on the so-called 'one-step' synthesis of purines[7] supports the imidazole pathway outlined in Fig. 1 as a mechanism for the synthesis of adenine from hydrogen cyanide under possible primitive Earth conditions.

Work is being carried out at present on the isolation of the three-carbon compounds assumed to be the precursors of the 4-amino-5-substituted imidazoles isolated, and also on the synthesis of other purines from hydrogen cyanide.

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J. Oró

Chemistry Department,
University of Houston,
Houston 4,
Texas.

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A New Phase formed by High-Pressure Treatment : Face-centred Cubic Molybdenum Monocarbide

The attempted preparation and retention of polymorphs dependent on pressure is an important area of high-pressure research. Some of these phases are found in Nature; others were unknown prior to the recent development of equipment suitable for research at high pressure and high temperature.

Although high-pressure phases are thermodynamically unstable at ambient conditions, a few have been retained by quenching to room temperature under pressure. High-pressure polymorphs found in the Earth's crust[1] include several minerals, for example, the pyroxene jadeite, $NaAlSi_2O_6$ (ref. 2); kyanite, Al_2SiO_5 (ref. 3); and diamond (ref. 4). Previously unknown phases are coesite[5], a form of SiO_2 with a higher density than quartz; borazon[6], a cubic form of boron nitride; and several compounds of the garnet type[1]. A number of high-pressure polymorphs of pure metals, which unlike the above compounds cannot be retained at ambient conditions, have been reported[7].

High-pressure experiments on the molybdenum–carbon system have revealed a new form for the monocarbide phase. The new form, designated α-MoC, has been retained to atmospheric pressure. Synthesis was achieved from both an equiatomic mixture of molybdenum and carbon and an equiatomic mixture of the compound Mo_2C and carbon at pressures and temperatures in the range of 40–70 kilobars and 1,800°–2,500° C. The X-ray diffraction pattern for α-MoC showed the first ten lines for a face-centred cubic structure. No additional lines were observed. The average lattice parameter was $a_0 = 4{\cdot}27$ Å. In addition to high-pressure experiments in which the only product was α-MoC, other experiments carried out at lower temperatures and pressures afforded evidence for all the phases reported[8] for the molybdenum–carbon system at atmospheric pressure. A summary of the lattice parameters of the various phases is given in Table 1.

Table 1. Composition and Dimensions of Phases in the Molybdenum–Carbon System

Composition	Symbol	Structure	X-ray data a_0(Å.)	c_0(Å.)	c_0/a_0	Molybdenum atoms/unit cell
Mo_2C	β	hexagonal closest† packing	3·002	4·724	1·574	2
MoC	γ	hexagonal	2·898	2·809	0·969	1
MoC	γ'	hexagonal	2·932	10·97	3·742	4
MoC	η	complex hexagonal closest† packing	3·00	14·58	4·86	6
MoC	α	face-centred cubic (sodium chloride)	4·27	—	—	4

Further experimental detail and a thermodynamic analysis of the effect of pressure on the equiatomic region of the molybdenum–carbon system will be published elsewhere.

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E. V. Clougherty
K. H. Lothrop
J. A. Kafalas*

Research Division,
Manufacturing Laboratories,
Cambridge,
Massachusetts.

* Present address: Lincoln Laboratory, Massachusetts Institute of Technology, Lexington, Massachusetts.

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Generation of Radicals in Pairs and the Kinetics of Radical Chain Reactions in Solution

Free radicals responsible for the propagation of chain reactions are commonly formed by the photochemical or thermal decomposition of an appropriate labile substance, and are, in such cases, inevitably produced in pairs. The restricted mobilities of radicals in solution have some special influence on the fate of these radicals, in that many pairs of radicals recombine before bringing about any reaction[1]. This effect,

sometimes known as the cage effect but more recently called[2] geminate recombination, has been widely discussed. A further consequence of the production of radicals in pairs has not, however, received consideration. Even if a free radical avoids recombination with its twin radical in the primary cage, the chain carrier will in general, at least in the earlier part of its life, lie closer to its twin than to another chain carrier. There is, therefore, a finite and sometimes appreciable probability of the mutual termination of geminate chains. The proportion of mutual termination of geminate chains must depend, on one hand, on factors controlling the rate of diffusion of the radicals in the medium and, on the other, the average relative distances of a radical to its twin and other radicals, and this will depend on the rate of decomposition of the initiating molecule.

The kinetic effects of recombination of geminate chains is best demonstrated by considering the extreme case where the various pairs of chains are so isolated that termination occurs exclusively by reaction between geminate chains. The rate of propagation of the reaction is then:

$$k_p\,(A)\,2n$$

where n is the number of geminate pairs of chains (expressed in terms of moles/l.), and k_p and (A) are respectively the rate coefficient for the propagation reaction and the molar concentration of the substrate:

$$n = I\tau$$

where τ is an average lifetime of a pair of geminate chains with respect to their mutual termination and I is the rate of decomposition of the initiator molecule. The rate of propagation of the reaction in the case of long chains is:

$$2\,k_p\,I(A)\tau$$

Since τ is determined only by diffusion factors, the rate of reaction is proportional to the first power of the rate of initiation. Normally in reactions in which chains terminate in bimolecular processes the rate of propagation depends on the square root of the rate of initiation[3].

In general there will be competition between the geminate chain termination and normal bimolecular termination, in which case, assuming a stationary state in the overall concentration of radicals (R):

$$\mathrm{d}(R)/\mathrm{d}t = 0 = I - 2k_t(R)^2 - 2(R)/\tau$$

where k_t is the rate coefficient for the bimolecular reaction between radicals of non-geminate chains and τ is now the mean lifetime of chains which terminate geminately. The rate of propagation of the chain reaction is:

$$k_p(R)(A)$$

The rate of propagation will, therefore, be a complicated function of the rate of initiation, but over a restricted range it may appear to depend on a power of I that lies between one-half and one.

In most systems the exponent of the rate of initiation in the rate equation is close to one-half[3], and in these cases geminate chain termination must be unimportant. However, systems are known where the exponent is significantly higher, notably the later stages of radical polymerization[4,5]. Among effects that can formally lead to first-order dependence on I are degradative transfer[6] and trapping of radicals[7]. In the former case it is difficult to visualize an appropriate reaction that is operative only at high conversion, and this, together with the observed electron-spin resonance spectra of trapped radicals[8,9] at very high conversions, has given support to the second explanation. This explanation is itself not without difficulties as the exponent begins to rise relatively early in the polymerization when the medium is far from rigid, and small radicals, particularly primary radicals, termination by which leads to an exponent less than one-half, are still mobile. Geminate chain termination can account formally for high-conversion kinetics and at the same time offers a plausible physical model in that the diffusing apart of growing geminate radicals must become severely restricted as the gel structure develops.

P. E. M. Allen
C. R. Patrick

Chemistry Department,
The University,
Birmingham, 15.

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Integration with Ionization Detectors in Gas Chromatography

The ionization current of the hydrogen flame ionization detector consists of a standing current and a current depending on the concentration of organic components emerging from the column. The standing current i_0 is constant when carrier gas velocity and column temperature are constant throughout the experiment. The ionization current due to organic compounds separated on the chromatographic column is a variable and will be denoted by $i(t)$.

The ionization current may, to some extent, depend on constructional parameters, but the voltage

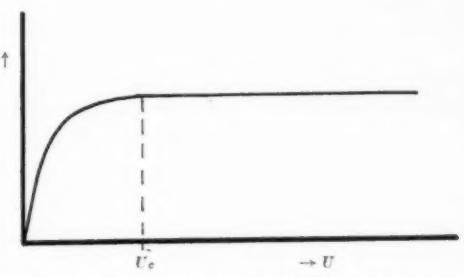

Fig. 1. Voltage-current characteristic of an ionization detector

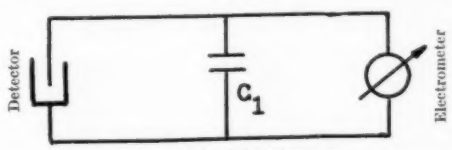

Fig. 2. Principle of the integrator

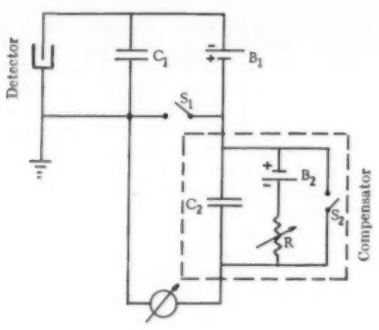

Fig. 3. Integrator with compensation. The values of the batteries, capacities and resistance given are approximate and may be varied with the operating conditions. C_1, 1,000 pF.; C_2, 100 μF.; B_1 and B_2, 90 V.; R, 100 $M\Omega$

across the electrodes has very little influence on the ionization current so long as the potential difference exceeds the value U_c in Fig. 1[1,2]. Therefore the usual constant-voltage source may be replaced by a condenser C_1 (Fig. 2).

During the experiment this condenser will be discharged and its potential drop over the time interval t_1–t_2 will be given by:

$$\Delta U = -\frac{1}{C_1}\int_{t_1}^{t_2}(i_0 + i(t))\,dt \qquad (1)$$

where C_1 is the capacity of the condenser.

When the detector response is linear with concentration, $-\frac{1}{C_1}\int_{t_1}^{t_2} i(t)\,dt$ will be proportional to the amount of an organic compound leaving the chromatographic column, while $-\frac{1}{C_1}\int_{t_1}^{t_2} i_0\,dt$ will be equal to $-\frac{i_0}{C_1}\cdot(t_2 - t_1)$ if the standing current can be considered as a constant.

Leakage of detector and condenser due to imperfect insulation will cause a current which is dependent on the voltage. This leakage, however, can be made small and almost independent of the voltage if ΔU is kept small as compared with U and very good insulation is being used. The leakage is then to be considered as part of i_0.

As one is only interested in $-\frac{1}{C_1}\int_{t_1}^{t_2} i(t)\,dt$, it is essential to separate the two parts of the ionization current. This can be done by using the arrangement shown in Fig. 3, where the standing current is compensated by charging a second condenser C_2 at a rate corresponding to that at which the voltage across C_1 decreases due to the standing current.

If the signal of the electrometer is registered on a potentiometric recorder, the initial conditions can be established again by operating the switches S_1 and S_2 simultaneously through the end contact of the recorder.

The integrator may be used, in principle, with any other ionization detector having a current-voltage characteristic as shown in Fig. 1, such as the argon detector[3].

P. M. E. M. van der Grinten
A. Dijkstra

Central Laboratory,
Staatsmijnen Geleen,
The Netherlands.

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BIOCHEMISTRY

Non-Random Occurrence of Amino-Acid Replacements

One approach to elucidating the genetic code is to study its manifestations in the primary structure of proteins. Of particular interest in this respect are amino-acid replacement regions, that is, those few positions in a protein chain where 'identical' proteins isolated from different species differ in amino-acid composition, differences presumably arising from mutations in the underlying genetic apparatus[1]. For example, insulin *A* chains isolated from beef, rabbit, whale, etc., have identical amino-acid sequences except at positions 8–10, where certain differences in amino-acid composition occur[2].

Sufficient data are now available so that we can begin to characterize amino-acid replacements to some degree, and hopefully then begin to understand more about the nature of the underlying genetic coding. The first characteristic of amino-acid replacements to note is that replacements resemble burst errors; that is, given that one replacement (error) has occurred, there is a greatly increased probability that another replacement (error) will occur at an adjacent position in the protein chain. Examples of this are the 8–10 positions in the insulin chain already referred to and the 32–39 positions in the adrenocorticotropin molecule[3]. It is not possible to say whether the replacements in such a region all occurred simultaneously, but this seems a possibility. Recent experiments on chemically induced mutants in tobacco mosaic virus could help to elucidate this point[4]. A second characteristic of amino-acid replacements is shown in Table 1. Here amino-acids are listed in order of their abundance in the proteins which contain known replacements. From columns *A* and *B* it is evident that there is no striking tendency for certain amino-acids to be preferred over others, either immediately following replacement regions or actually involved in them. (The terms 'following' and 'preceding' are defined so that a given amino-acid 'precedes' amino-acid further than itself from the amino end of the molecule. A 'replacement region' is any contiguous section of a protein chain, one or more amino-acids in length, where replacements have been recorded at all positions within the region.) In contrast, there is a strong tendency for amino-acid replacement regions to be preceded by one of the four amino-acids glycine, proline, tyrosine, or cysteine. None of these four amino-acids is in the top 30 per cent in abundance, and only glycine is in the top 50 per cent in abundance. One would expect, on a

Table 1. Frequency of occurrence of amino-acids (*A*) in, (*B*) immediately following, and (*C*) immediately preceding replacement regions; data are those collected by Ycas (ref. 8). Amino-acids are listed according to order of abundance in the proteins which contain replacement regions. Only those replacements which do not occur at either terminal position of a protein chain are tabulated

Amino-acids in order of abundance	*A* Involved in replacement region	*B* Immediately following replacement region	*C* Immediately preceding replacement region	$\frac{C}{A+B}$
1. glu	11	3	1	0·07
2. ala	9	3	0	< 0·1
3. asp	5	1	1	0·2
4. ser	10	0	0	< 0·1
5. val	4	1	1	0·2
6. lys	7	1	0	< 0·2
7. gly	3	2	5	*1·0*
8. leu	5	1	0	< 0·2
9. thr	3	0	1	0·3
10. cys	0	2	2	*1·0*
11. pro	3	1	5	*1·3*
12. arg	3	1	0	< 0·3
13. tyr	0	1	3	*3·0*
14. phe	1	0	0	—
15. his	1	1	0	< 0·5
16. ilu	3	0	0	< 0·3
17. met	0	1	0	—
18. try	0	0	0	—

random-choice basis, one of these four amino-acids to precede replacement regions in one-fifth to one-quarter of cases, but the observed frequency is four-fifths. In summary, then, amino-acid replacements are possibly burst errors and have a high probability of being preceded by one of four other amino-acids.

If one attempts to find other genetic phenomena having characteristics similar to those of amino-acid replacements, at least two are found. One is the occurrence of genetic 'hot spots' in the saturation genetic mapping studies on T_4 bacteriophage[5]. These 'hot spots' are 'points' in a T_4 genetic map where about 100 mutants are recorded. At other points in such a map only one or at most a few mutants have been found. This phenomenon recalls the likelihood of amino-acid replacements to occur at certain regions in a protein chain. (There are about ten replacements recorded in the various examples of the insulin *A* chain, all at positions 8–10 (ref. 2).)

The second genetic phenomenon in ways resembling amino-acid replacements again arises in connexion with the T_4 genetic map. Specifically, certain mutations, termed 'deletions', occupy a considerable distance on the linear genetic map[5]. There is no evidence to our knowledge that a segment of the bacteriophage genome has actually been removed in these cases. It is evident from the above discussion that some 'deletions' could also be burst errors.

In that genetic 'hot spots' and 'deletions' both have characteristics similar to those of amino-acid replacements, we should attempt a unified explanation for all three. Recently, Golomb has suggested a coding scheme which appears to account for genetic 'hot spots'[6]. On this scheme the origin of a hot spot is in the make-up of certain code words in an error-correcting, 'biorthogonal', comma-free, nucleotide sextuplate code. In that this code is comma-free (therefore, no influence of one code word on neighbouring ones), it does not explain either of the characteristics associated with amino-acid replacements (burst error aspect or high probability of following one of four amino-acids), so it is not a unified explanation of all three phenomena.

It seems difficult in the present state of our knowledge to present an explanation of these characteristics of amino-acid replacements, but attempts to do so will force us to consider many genetic coding mechanisms now given little or no serious attention. For example, the question of whether an amino-acid template includes an adjacent amino-acid residue in addition to nucleotides is raised. The possibility that an error in protein synthesis can result in a mutation is also suggested. (Stent *et al.* have been collecting evidence that replication of deoxyribonucleic acid intimately involves very specific protein synthesis[7].) Perhaps overlapping degenerate coding schemes should be investigated. These results also indicate that genetic error-correcting mechanisms may exist in the cell.

Recent attempts by me to develop a nucleotide triplet code based on the composition of the RNA viruses have given a suggestion as to how an explanation of these phenomena might be developed, and, at the same time, become reconciled in part with existing concepts of genetic coding[9,10]. When such a nucleotide triplet code was devised, it turned out that most of the triplets could be included in a comma-free system. However, a few of them could not fit such a system; notable among the exceptions were the triplets corresponding to the amino-acids proline, tyrosine, and cysteine (that is, three of the four amino-acids which precede replacement regions with a high probability). The cardinal characteristic of a comma-free code is that 'displaced words', words formed from adjacent letters in two adjacent coding words (for example, if *ABC* and *DAB* were two adjacent coding words, then *BCD* and *CDA* are displaced words), are never coding words also. This suggests that associated with the amino-acids having a high probability of preceding replacement regions are nucleotide coding words capable of producing some displaced words which themselves are coding words. For example, overlap between *CCC* and *CXX* produces the displaced word *CCC*, which is also a coding word. Now, where the displaced words, like that in the example, are the same as one of the code words producing them, we might have a mechanism whereby displaced words used mistakenly by the cell as code words initiate burst errors, resulting in replacement regions which have a high probability of being preceded by certain amino-acids. Such a mechanism as briefly sketched here would, of course, necessitate postulating also cellular mechanisms which cope with, eventually terminate, such a burst error without interrupting synthesis of a protein chain, and a feedback mechanism whereby an error in certain protein synthesis eventually produces a genetic error (mutation). (The previously cited work of Stent *et al.* makes this suggestion not too improbable.) Though these concomitant postulates appear somewhat extreme at present, it seems as though an explanation of errors producing amino-acid replacements may force us to think in now unfamiliar terms.

C. R. Woese

General Electric Research Laboratory,
Schenectady, New York.

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A Simplified Method for separating Amino-acid Mixtures from Protein Hydrolysates on One Ion-Exchange Column during Preparation

Most of the methods used for the separation of mixtures of amino-acids are based on the work of Moore *et al.*[1-3]. Separation is achieved either on two columns, acidic amino-acids being separated on a 'Dowex' 1 anion-exchange column and the basic and neutral amino-acids on a 'Dowex' 50 cation-exchange column or by chromatography on one column on 'Dowex' 50 in the H-cycle, elution being carried out with citrate phosphate buffer solutions. Hamilton and Anderson[4] recently described a semi-automatic method for these separations containing several improvements of the original process. These methods ensure good separation of amino-acids; the eluates, however, contain either the hydrochlorides of the amino-acid and hydrochloric acid or considerable amounts of salts, which are difficult to remove. An efficient method for the separation of amino-acids should permit separation in a small volume of the elution reagent in a short time; the buffers used should be removable by a simple method. Wilcox *et al.*[5] used volatile buffers for the separation of aspartic and glutamic acid on an anion-exchange column. Vaněček *et al.*[6] and Tomášek[7], used volatile buffers successfully for the separation of peptides on 'Dowex' 50.

In connexion with our work[8,9] on the preparation of amino-acids-^{14}C from proteins containing carbon-14 obtained from a photosynthesizing organism, we elaborated a method fulfilling the requirements mentioned here.

Separation of the amino-acids from the protein hydrolysate was carried out on one 'Dowex' 50 4×200–400 mesh column. A column of $2{\cdot}0 \times 150$ cm. was operated at 37° C. Amounts of amino-acids of 600 mgm. were separated on this column. The ion-exchange column was prepared according to Tomášek[7], that is, from the H-cycle transferred to the pyridine cycle and equilibrated with a 0·1 *M* pyridine formate buffer solution of *p*H 3·1. Elution was carried out by the gradient system. The buffers used are listed in Table 1. The effluent was collected by an automatic fraction collector in 5–20 min. intervals and at an effluent-rate of 7·5 ml./10 min. The fractions were identified by chromatography and autoradiography and the radioactivity of the suitably combined fractions determined. A typical result of the separation is given in Fig. 1. The amino-acids were obtained in about 1,200 ml. of the eluate, the total volume being 2·5–31. All the amino-acids were obtained in pure form except serine–proline and alanine–glycine, which were eluted in pairs and have to be separated chromatographically. The pure amino-acids were isolated in the free form by repeated evaporation of the aqueous solution *in vacuo*. The yield of pure amino-acids amounted to 75 per cent of the radioactivity of the hydrolysate. The method is easily reproducible.

Table 1. Composition of the Buffer Solution

Buffer	Pyridine (mole)	*p*H	*p*H adjusted with
1	0·1	3·1	formic acid
2	0·2	3·1	formic acid
3	0·2	4·4	acetic acid
4	1·0	5·1	acetic acid
5	2·0	5·1	acetic acid

As this method is simple to perform and gives good separation results, it should be generally applicable to the separation of amino-acids from protein hydrolysates.

J. Liebster
K. Kopoldová
M. Dobiášová

Radioisotope Laboratories,
Biological Institute,
Czechoslovak Academy of Sciences,
Na Cvičišti 2, Prague, 6.

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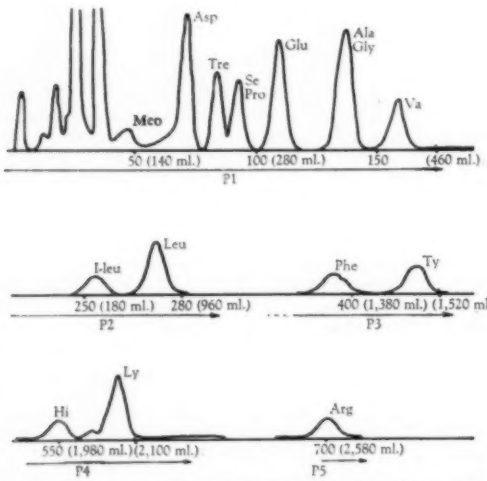

Fig. 1. *X*-axis, number of fractions (ml. of eluate); *y*-axis, relative radioactivity in c.p.m.

Oxidative Demethylation and Carcinogenicity of 4-N-Dimethylaminoazobenzene Derivatives

Recent work on the hepatocarcinogenic amino-azo compounds has shown that the presence of at least one N-methyl group and the formation of a firmly bound compound between metabolites of the azo compound and rat-liver proteins are probably necessary for the strong activity[1]. The biochemical behaviour of N-methyl groups with respect to the protein-bound polar dye formation has not yet been elucidated satisfactorily, because of the structural complexity and very little amounts of polar dye obtained. We showed that the N-^{14}C-methyl group of 4-N-^{14}C-methylaminoazobenzene was incorporated almost quantitatively into the polar dye[2]. The N-methyl group of 4-N-methylaminoazobenzene (MAB), being one of the most reactive sites and rapidly demethylated to split formaldehyde, appears to be responsible for the polar dye formation in an active metabolic intermediate.

N-Demethylation, which is one of the major metabolic pathways of 4-N-dimethylaminoazobenzene

(DAB), MAB and their derivatives[3], is carried out by liver microsomes and requires both reduced triphosphopyridine nucleotide and free oxygen[4], though a clear picture of its precise mechanism is not yet established. Unpublished investigations in this laboratory have shown that the system consisting of the iron chelate of ethylenediaminetetraacetic acid (EDTA), ascorbic acid and free oxygen, which was established as a hydroxylase model by Udenfriend[5], is also suitable for the demethylase model. The present communication demonstrates that this system demethylates ring-methylated derivatives of DAB to their MAB analogues, and the rate of reaction is correlated with the π electron density[6] at N-dimethylamino nitrogen.

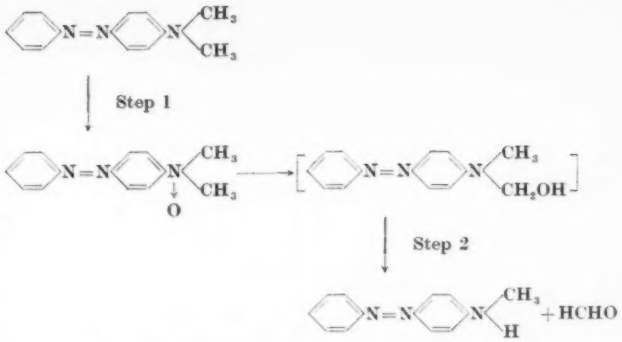

Fig. 2. Proposed mechanism of oxidative demethylation of DAB

The reaction system given in Table 1 was incubated in the metabolic shaker, designed for the purpose, for 4 hr. at 40° in an atmosphere of oxygen (Po_2 = 785 mm. mercury). The reaction was terminated by adding 50 ml. of 0·5 per cent sodium bicarbonate solution and the dyes were extracted three times with chloroform. Chloroform was evaporated under reduced pressure, and the residue dissolved in light petroleum (boiling point 60°–80°) was subjected to chromatography using an alumina column, in order to separate the demethylated products from the substrate. Demethylation, as measured by the amounts of MAB derivatives produced, depended on *p*H and temperature. As the reaction did not occur in the absence of oxygen, the system may function as an oxygenase.

Table 1. Demethylation of DAB Derivatives

Compound	Rate of demethylation (10^{-6} moles/4 hr.)	Carcinogenicity (ref. 3)
4′-Me-DAB	1·42 ± 0·11	1
3′-Me-DAB	1·12 ± 0·08	10 ~ 12
2′-Me-DAB	1·41 ± 0·08	2 ~ 3
DAB	1·18 ± 0·08	6
2-Me-DAB	1·36 ± 0·04	0
3-Me-DAB	0·843 ± 0·043	0

The system contained $1·5 \times 10^{-5}$ moles of azo dyes, $1·5 \times 10^{-5}$ moles ferrous ion as ferrous ammonium sulphate, $3·0 \times 10^{-5}$ moles EDTA, $3·0 \times 10^{-4}$ moles ascorbic acid, and $10 \times 1·0^{-3}$ moles phosphate buffer, *p*H 7·3, in a total volume of 50 ml. (30 per cent acetone solution).

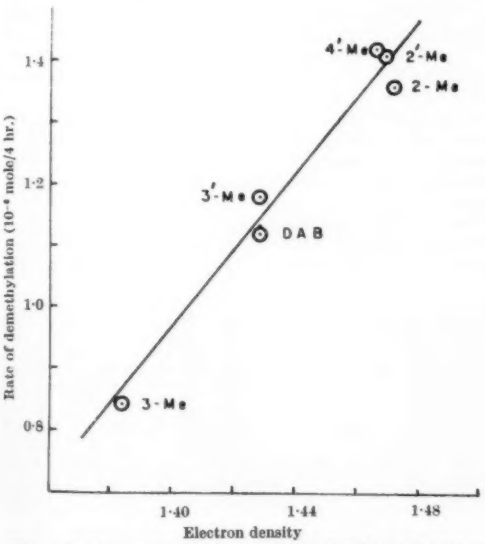

Fig. 1. Relation between the rate of demethylation and total π electron density at the dimethylamino nitrogen of DAB derivatives

The rate of demethylation was shown to be satisfactorily correlated with the π electron density at N-dimethylamino nitrogen, but not with the carcinogenicity (Fig. 1 and Table 1). However, it is interesting to see that strong carcinogens such as DAB and 3′-methyl DAB show moderate values of the reaction-rate. 4-N-Dimethylaminoazoxybenzene amino-oxide, which was assumed to be an analogue of the active intermediate, could be demethylated quickly to MAB in the presence of ferrous ion alone, and formaldehyde was detected. With the same conditions, 4-N-dimethylaminoazoxybenzene was resistant to the oxidative demethylation. The active oxygen thus appears to attack first the nitrogen atom of the dimethylamino group (electronegative centre), and forms the unstable amino-oxide, which seems to be rearranged in turn to split off formaldehyde (Fig. 2). According to the relation shown in Fig. 1, step 1 in Fig. 2 may determine the rate.

We thank Prof. H. Terayama, University of Tokyo, for his advice and interest in our work.

Morizo Ishidate
Akira Hanaki*

Faculty of Pharmaceutical Science,
University of Tokyo.

* Present address: National Institute of Radiological Sciences, Chiba, Japan.

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Chromatographic Fractionation of Hog Thyroglobulin

Derrien *et al.*[1,2] have purified thyroglobulin, the main protein component of thyroid, in a native state, and stated that their preparation was ultracentrifugally as well as electrophoretically homogeneous. However, since the iodine content of thyroglobulin varies with preparations, especially with

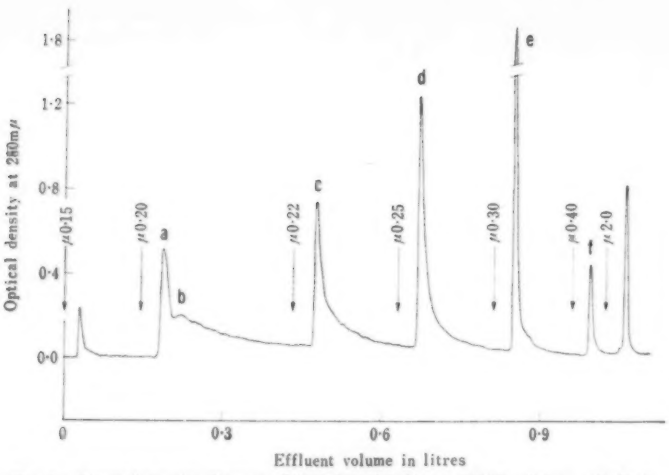

Fig. 1. Chromatographic diagram of hog thyroglobulin on *DEAE*-cellulose. Vertical arrows indicate the point of application of eluants. $1/N$ for the peaks are: *a*, 25; *b*, 27; *c*, 30; *d*, 34; *e*, 39; *f*, 35

When stepwise elution was carried out according to the elution schedule indicated by arrows in Fig. 1, there appeared several thyroglobulin peaks (fractions $a-f$) with an increase in the concentration of sodium chloride in the buffer. Other small peaks, one which emerged on washing the column and one with elution at ionic strength 2·0, were due to minor impurities; the latter component was found by means of its ultra-violet absorption spectrum and chemical analysis to be ribonucleic acid. The total apparent recovery was 87 per cent as determined by ultra-violet absorption at 280 mμ. Re-chromatography was almost satisfactory, but fractions eluted in lower ionic strengths had a tendency to emerge on re-chromatography at a higher ionic strength than in the initial chromatography if the ratio of the load of protein to the amount of *DEAE*-cellulose was lower on re-chromatography. In a gradient elution experiment, too, heterogeneity of thyrogobulin was indicated, but the separation was poorer, as reported by Ingbar *et al.*[6].

different physiological states of animals from which it is prepared, it is reasonable to assume that thyroglobulin is not a single protein, but consists of proteins iodinated to different degrees.

The following evidence, obtained in our laboratory (Ui, N., and Tarutani, O., work to be published), also indicates inhomogeneity of thyroglobulin. Ultracentrifugal analyses showed, in agreement with the findings of other investigators[3], the presence of a greater or lesser amount of a faster sedimenting component ($S^0_{20,w} = 28$ S.) in the preparations. This component, tentatively named *F*-component, could not be distinguished from the main component ($S^0_{20,w} = 19$ S.) with respect to solubility characteristics. In moving-boundary electrophoresis, although the preparation always showed a single boundary, the heterogeneity was indicated by the 'reversible boundary-spreading' technique[4], even with a preparation essentially free from the *F*-component which was occasionally obtained.

In view of these findings, we have attempted to fractionate thyroglobulin. The present communication describes the result of cellulose ion-exchange chromatography and some properties of the fractions obtained.

Thyroglobulin used in this work was prepared from hog thyroid glands according to a modified method of Derrien *et al.*[1], in which fractional salting-out between 1·55 and 1·75 *M* ammonium sulphate was performed at a low temperature instead of the 20° C. in the original method.

In Fig. 1 is shown a typical chromatographic diagram. In this experiment, about 130 mgm. of thyroglobulin dissolved in a phosphate-sodium chloride buffer (NaCl: 0·05 *M*) of *p*H 6·5 at ionic strength (μ) of 0·15 was applied to a column of *DEAE*-cellulose[5] (1·5 cm. × 30 cm.) equilibrated with the same buffer. The column was operated at a rate of approximately 20 ml./hr. The effluent was collected in 3-ml. fractions, and the optical density at 280 mμ was determined.

Chemical analyses of these chromatographic fractions showed that the ratio of iodine to nitrogen increased steadily from fraction *a* to fraction *e* with an increase in the ionic strength of the eluant. The ratio expressed as μgm. iodine/mgm. nitrogen for each peak is given in the legend of Fig. 1. In an ultracentrifuge (Fig. 2), fractions *a*, *b* and *c* showed a single boundary corresponding to the main component of the original preparation, while *F*-component was found to be concentrated in fractions *d*, *e* and *f*, especially in fraction *e* (about 30 per cent). Fraction *e* contained, besides ordinary *F*-component (F_a), an additional component with a higher sedimentation coefficient (F_β).

Attempts to obtain pure *F*-component have been, so far, unsuccessful, the purity being no more than 62 per cent after repeated chromatography. However, it was considered from iodine analysis of these preparations that *F*-component would be iodo-protein(s). Further work is necessary to confirm this.

Although these experiments were carried out on a preparation derived from a number of thyroid glands, a similar chromatographic diagram was obtained with a preparation obtained from a single gland.

On the basis of these observations it is suggested that hog thyroid contains a large number of iodoproteins, which differ in the degree of iodination

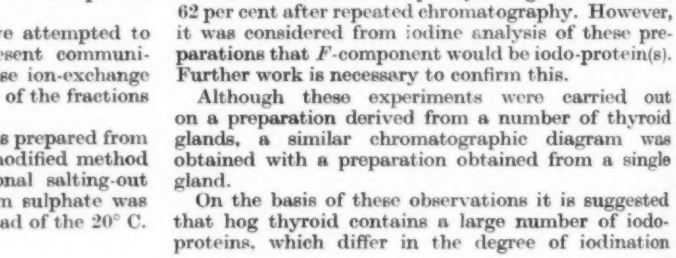

Fig. 2. Ultracentrifugal patterns of thyroglobulin fractions. (1) Original thyroglobulin preparation; (2) fraction *a*; (3) upper, fraction *d*; lower, fraction *e*. Sedimentation proceeds from right to left

and in other properties. It should also be noted that, in spite of this heterogeneity, no iodine-free component was found in thyroglobulin.

Further work is now in progress, and the results will be published elsewhere together with details of the present experiments.

NOBUO UI
OSAMU TARUTANI
YOICHI KONDO
HIROKO TAMURA

Institute of Endocrinology,
Gunma University,
Maebashi,
Japan.

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Selenomethionine as an Antioxidant

EVIDENCE has accumulated indicating that: (1) vitamin E functions, at least in part, as a lipid antioxidant; (2) unrelated substances having antioxidant activity can alleviate or prevent some of the symptoms of vitamin E deficiency[1]; (3) selenium compounds also can alleviate or prevent some of the symptoms of vitamin E deficiency[2]; (4) the ingestion of selenium oxide renders tissues less susceptible to oxidative attack[3]; (5) some seleno-amino-acids have antioxidant activity in aqueous lipid emulsions[4]. The experiments reported here were undertaken to determine whether seleno-amino-acids could act as antioxidants in anhydrous fats and oils.

Denison and Condit[5] had first shown that organic selenium compounds were powerful antioxidants in lubricating oils. Woodbridge[6] has summarized the literature dealing with selenium compounds as anti-oxidants in rubber and lubricating oils and contributed information on their effectiveness in squalene.

Most of the experiments were run with selenomethionine, one sample of which was obtained from E. Painter[7], and a second from Y. Natori. The latter sample was pure by paper chromatography. Similar results were observed for both samples. Triolein was obtained from the Hormel Foundation. Lard was a commercial sample containing no added antioxidants. Menhaden oil was a purified sample obtained from the Bureau of Commercial Fisheries.

Antioxidant activity was estimated by the gain-in-weight method described previously[8]. Covered beakers containing the oil samples were incubated in constant-temperature draught ovens. Once daily they were removed from the oven, tested for rancidity by odour, allowed to cool to room temperature, weighed and returned to the oven. The end of the induction period was indicated by a gain in weight and also by the development of rancidity.

Peroxides were determined by iodimetry, essentially according to the A.O.C.S. method Cd 8–53 but adapted for a sample size of 200 mgm. The containers were kept filled with nitrogen during the one minute reaction period with potassium iodide.

Table 1. EFFECT OF SELENOMETHIONINE ON INDUCTION PERIOD OF TRIOLEIN, LARD, MENHADEN OIL*

Additive	Amount (μM/gm.)	Induction period (days)
Triolein (60°):		
None	—	5, 5
Selenomethionine	1	5
	2·5	9
	5	57
	10	31
	25	> 355
Methionine	10	8
	25	9
Lard (60°):		
None	—	1, 2
Selenomethionine	10	6, 6
	25	> 240, > 240
	50	> 240, > 240
Methionine	10	1, 1
	25	1, 1
Menhaden oil (50°):		
None	—	1, 1
Selenomethionine	10	1, 1
	25	1
	50	1

* Fat samples which were held for months at 60° without becoming rancid did, however, develop a garlic odour, and gradually became more viscous.

As shown in Table 1, selenomethionine was ineffective in the various substrates at levels below 5 μM/gm.; at higher concentrations it gave remarkable stabilizing effects with lard and triolein, but was ineffective in menhaden oil (a highly unsaturated fish oil, iodine value 160). The protection afforded the more saturated fats was not uniformly demonstrable, and it was noted in most instances that oxidation, as measured by gain in weight, proceeded for a few days to 0·25–0·75 per cent of the total weight, and then came to an abrupt stop. No changes then occurred for several months. Parallel runs with methionine did not show similar behaviour. Other seleno-amino-acids, namely, selenocysteine, phenyl selenoglycine, selenohomocysteine, and phenyl selenomethionine, also acted as antioxidants when added in relatively large amounts, but they were not as exten-

Table 2. PEROXIDE CHANGES IN LARD INCUBATED AT 60° WITH AND WITHOUT ADDED SELENOMETHIONINE AND HYDROPEROXIDE

	Additive	Amount (μM/gm.)	Original peroxide value	Time (hr.)	Final peroxide value
Run 1*	None	—	100	44	435
	Se-methionine	10	0	44	104
				138	77
	Se-methionine	10	100	74	122
				138	76
	Se-methionine	25	0	74	80
				138	41
	Se-methionine	25	100	74	44
				138	15
Run 2†	None	—	0	24	305
				48	380
	None	—	200	24	445
				48	370
	Se-methionine	25	0	24	71
				48	63
				72	50
	Se-methionine	25	200	24	91
				48	69
				72	54
	Methionine	25	0	24	250
				48	245
				72	293
	Methionine	25	200	24	429
				48	485
				72	370

* Hydroperoxide added as oxidized safflower oil methyl esters.

† Hydroperoxide added as menthane hydroperoxide.

sively studied. Quantitation with the selenoamino-acids was rendered difficult by the heterogeneity of the system; none was completely in solution in the substrates used.

Changes in peroxide value were followed in lard samples with and without added selenomethionine and also with and without added peroxides. The results, shown in Table 2, indicate that in the presence of selenomethionine the peroxide values did not rise so high and tended to decrease with time, more so than when either methionine or no additional additive was present. In this sense selenomethionine appeared to be able to decompose peroxides.

The results with selenomethionine differed from those observed with other antioxidants in: (1) they appeared to have no effectiveness in low concentrations; (2) the protection even in large amounts could not be uniformly demonstrated; (3) no protection was afforded to a highly unsaturated oil; (4) some oxidation appeared to occur before the antioxidant effect appeared. These observations may tentatively be explained by assuming that selenomethionine is not itself an antioxidant but that it is oxidized to a derivative which is. Such systems have been described for thio- and dithio-compounds by Barnard *et al.*[9], and for seleno-compounds by Woodbridge[6]. In the former case the active antioxidants are thought to be the derived sulphoxides, and thiosulphinates.

It is reasonable to suppose that the reactions which lead to the formation of a sufficient amount of a powerful antioxidant so that oxidation stops would be of a complexity such that only at times could this consummation be reached. Thus, if a highly unsaturated oil formed peroxides rapidly, the intermediate antioxidant might be further oxidized before it could stabilize the reaction.

These results give further suggestive evidence that some of the tocopherol-like physiological properties of selenium compounds may be mediated through an antioxidant action[4]. Perhaps the lack of parallelism in their physiological effects is due not only to the differences of availability to different cells of tocopherols compared with seleno-compounds but also to the requirement that specific selenium derivatives, which occur or are synthesized in ways not yet understood, are required to achieve the antioxidant state.

H. S. Olcott
W. Duane Brown
J. Van der Veen

Institute of Marine Resources,
Department of Nutritional Sciences,
University of California,
Berkeley.

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Occurrence of Mevalonic Acid in Carrots

Although tracer studies in a number of carotenogenic systems[1,2] have conclusively proved that mevalonic acid is an effective precursor of carotenes, the presence of mevalonic acid has not been demonstrated in any of such systems. In this laboratory an attempt has been made to isolate mevalonic acid from carrots. Previous work[3,4] has shown that cell-free extracts of carrots can synthesize carotenes from acetate and mevalonic acid.

100 gm. (wet weight) of freshly collected carrots were minced and placed in 100 ml. phosphate buffer (0·1 *M*, *p*H 5·8) for about 2 min. in a Waring blender to prepare the homogenate. From the filtered homogenate the proteins were removed by treatment with trichloracetic acid and the supernatant, after neutralization, was concentrated *in vacuo* to about 60 ml. For isolation of mevalonic acid the concentrated material was acidified with 8 ml. of 10 *N* sulphuric acid, then extracted with ethyl ether for 8 hr. and the extract treated according to the method of Lynen and Grassl[5]; finally mevalonic acid was determined by reaction with hydroxylamine at *p*H 7·0 and subsequent colorimetric measurement of the resultant hydroxamic acid by formation of a complex with ferric salts. The quantitative range of mevalonic acid present in carrots varied from 2 to 4 μmoles/100 gm. of carrots (wet).

The dibenzylethylenediamine (DBED) salt of isolated mevalonic acid was prepared according to the procedure described by Hoffman *et al.*[6]; a crystalline salt was obtained which melted at 115° (114–119°).

For further confirmation the mevalonic acid DBED salt was dissolved in minimum amount of water, the *p*H was adjusted to 10 and the free amine was extracted with ethyl ether. The alkaline solution was readjusted to *p*H 6·5–7, and then, after concentration, portions were chromatographed according to the method of Yokoyama *et al.*[2] and the spot of mevalonic acid was identified by spraying with bromophenol blue.

The compound isolated was also confirmed as mevalonic acid by identifying the hydroxamate derivative which was chromatographed by using butanol saturated with water as the solvent system as reported by Knauss *et al.*[7]. The spots were made visible by spraying with 5 per cent $FeCl_3.6H_2O$ in 90 per cent ethanol and 0·1 *N* hydrochloric acid.

In view of the recent findings of Braithwaite and Goodwin[8] that mevalonate is more efficiently incorporated than acetate into carotenes of carrots, the occurrence of mevalonic acid in carrots makes the carrot system a valuable tool for further work.

V. V. Modi
D. K. Patwa

Biochemistry Department,
Faculty of Science,
M.S. University of Baroda,
India.

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HISTOCHEMISTRY

Tissue Dilution Artefact; a Re-interpretation of Variations in Levels of Succinic Dehydrogenase during Chemical Carcinogenesis

RECENT biochemical studies on succinic dehydrogenase in livers of rats fed with the liver carcinogen *p*-dimethylaminoazobenzene (DAB) for periods of up to 150 days have confirmed the well-established phenomenon that the level of this enzyme generally falls as feeding progresses (Table 1). Samples of tissue from each liver lobe were taken for histological and histochemical investigations, and the remainder was homogenized. Succinic dehydrogenase was estimated to within an accuracy of ± 10 per cent by the anaerobic methylene blue technique, basing the Q_{MB} values on dry weight. For histochemical purposes the tissue was frozen[1], stored for not longer than a week, and cut at $-20°$ C. in a refrigerated microtome. Succinic dehydrogenase was detected histochemically by incubating frozen sections in a neotetrazolium–succinate buffer system[2]; in addition, the periodic acid–Schiff method[3] was used to stain glycogen, while phospholipid was stained by the acid hæmatin method[4].

The histochemical results indicated that the succinic dehydrogenase activity of the liver cells was high in liver from rats fed on a complete diet or on a low-protein methionine-supplemented diet[5] with or without DAB, and that in the course of feeding this activity per cell did not alter detectably. In contrast, normal bile duct epithelium had a low activity, while connective tissue gave no apperent reaction ; as the period of feeding increased, the proportion of these less or non-reactive components became greater, and the gradual fall in Q_{MB} was qualitatively matched by a reduction in the proportion of liver cells, which histochemically still reacted in a manner comparable with normal liver cells. Sections have also been stained with hæmatoxylin and eosin, while the periodic acid–Schiff and acid hæmatin stains have provided useful information in distinguishing cell types. The latter, in particular, differentiates the weakly staining cells of bile duct and connective tissue in contrast to the strong reaction of liver cells.

When rats were allowed to remain on diet for 200 days, the Q_{MB} values, even of tissue samples selected from the same lobe, showed considerable variation. High Q_{MB} values, about 75–90 per cent of control values, were given by areas containing a high proportion of liver cells, though this proportion was not as high as in normal liver. Low values of Q_{MB}, 20–50 per cent of control values, were obtained from areas of liver composed principally of bile duct cells and fibrous tissue.

Daoust and Cantero[6], who studied the livers of Wistar rats, showed that as the period of feeding the carcinogen DAB was extended, so the relative proportion of liver cells decreased, while the proportions of connective tissue and bile duct cells increased. In these studies, rats of the August strain have been used, but the histology of these livers was qualitatively similar to that reported by Daoust and Cantero. Thus the proportion of liver cells containing a high level of succinic dehydrogenase fell, while the amount of much less active bile duct cells and inactive connective tissue increased ; the overall fall in succinic dehydrogenase was due, not to any change, malignant or otherwise, in any particular cell type, but rather to an alteration in the relative proportions of cell types. It is suggested that the term 'tissue dilution artefact' be used to describe this phenomenon.

Table 1. VARIATIONS IN VALUES OF Q_{MB} IN THE RIGHT ANTERIOR LOBES OF MALE RATS (AUGUST STRAIN) FED WITH THE LIVER CARCINOGEN DAB ON A LOW PROTEIN DIET

Period of feeding	Q_{MB} in right lobe (DAB)	Q_{MB} in right lobe (control)	DAB Q_{MB} as percentage of control values
No DAB		25 ± 4	
30 days	23	25	92
60 days	20	24	83
90 days	13	22	59
120 days	15	29	52
150 days	10	22	45

It is claimed then that this artefact may occur generally in biochemical studies of a tissue containing two or more cell types which may vary in proportion during the course of the experiment. A notable example of such investigations is that on livers of animals treated with liver carcinogens. In this kind of work the onus lies with the experimenter to demonstrate that biochemical comparisons reflect a true alteration in cell metabolism, and are not due to a change in the proportion of cell types of different metabolic activities. Rigid histochemical and histological checks become of the utmost importance in interpreting such biochemical results if invalid conclusions based solely on such data are to be avoided.

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G. R. N. JONES
L. BITENSKY
J. CHAYEN
G. J. CUNNINGHAM

Department of Pathology,
Royal College of Surgeons,
Lincoln's Inn Fields,
London, W.C.2.

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PHYSIOLOGY

Sub-fractionation of Human Serum Leucine Aminopeptidase

OUR earlier starch-gel electrophoretic zymograms[1], obtained with 1-leucyl-β-naphthylamide (LBNA) substrate, revealed a single leucine aminopeptidase (LAP) zone for human serum. The substitution of alanyl-β-naphthylamide (ABNA) substrate now consistently reveals, within the diffuse trail behind the primary zone, an additional less-intense zone (Fig. 1*a*), detectable also for some sera by LBNA. Occasional sera give three or more zones; and one chronic pancreatitis serum has given a remarkable zymogram with five zones which, when observed through a suitable blue filter (Dupont No. 9 plastic 'Varigam' filter), can be distinguished as eight zones (Fig. 1*b*).

Whereas some zymograms are distinctive because of this multiplicity of zones, others are distinctive

because of a single zone. In 21 determinations, ABNA substrate on 18 serum samples from 8 varied patients (run on 4 different days over a 3½-month period), the usual two zones were resolved in every instance. Conversely, in each of 5 determinations on 5 serum samples from one other patient (run on 3 different days along with the other patients' samples), only the one primary zone could be found. Although no correlation with diagnosis is at present justified in this case, the serum of this particular patient seems clearly characterized by a marked deficiency or absence of at least one common peptidase subfraction.

The electrophoretic sub-fractionation is accomplished (as previously described[1,2]) in 15 per cent starch gel, prepared in 0·03 *M*, *p*H 9·0, borate buffer (final gel *p*H 8·5); and a 6 V./cm. gradient for 6 hr. The gel strip (2·0 × 0·65 × 25 cm.) is then cut horizontally into two thin slices and incubated at room temperature in a substrate solution[3], prepared by mixing and dissolving: (*a*) 8·0 ml. of a 1 per cent solution of ABNA (or LBNA) in water; (*b*) 40·0 ml. of 0·2 *M tris*(hydroxymethyl)aminomethane buffer that has been adjusted to *p*H 7·1 with hydrochloric acid; (*c*) 160 ml. of water; (*d*) 120 mgm. of diazotized *o*-aminoazotoluene. The zymogram of orange red zones against an orange yellow background usually begins to develop within 15 min., although 2 hr. are allowed for full development. The gels are finally rinsed thoroughly with water and saturated overnight with glycerol, which prevents drying and preserves the zymograms.

Special clinical interest attaches to this new evidence that LAP is heterogeneous. An early optimism that increased LAP activity in serum may be diagnostic of pancreatic cancer[4] has been questioned by similar findings for non-malignant diseases[5,6]. (Similarly, while Burstone[7] has associated abnormal LAP increases in certain tissues with invasive cancer, Monis *et al.*[8] attribute such increases to non-specific inflammatory and proliferative processes.) But beyond the controversial significance of total serum LAP activity, it is now apparent that a zymogram or distribution pattern of this activity among several different LAP sub-fractions can be much more informative, perhaps because each sub-fraction independently reflects the condition of its own particular source tissues. This situation has been demonstrated for other enzymes, tissues and diseases, most recently by Wroblewski[9] for heart lactic dehydrogenase, which selectively increases in serum after myocardial infarct. On this background our present work on LAP zymograms will be extended with emphasis on pancreatic cancer, other pancreatic and liver diseases, pregnancy and other conditions showing unusual serum LAP activity.

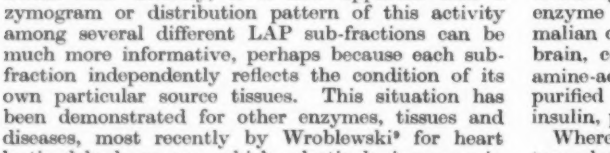

Fig. 1. Leucine aminopeptidase zymograms (ABNA substrate) of human serum (schematic). *a*, Typical pattern; *b*, a chronic pancreatitis pattern (observed through blue filter). The relative locations of the major protein zones (Smithies nomenclature) are noted below the zymograms

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Working independently of us, Sanford H. Lawrence, Perry J. Melnick and Harry E. Weiner (private communication) have also found two or more LAP zones for human serum.

Clyde A. Dubbs

Veterans Administration Center and
University of California Medical Center,

Charlotte Vivonia
Jean M. Hilburn

University of California Medical Center,
Los Angeles, California.

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Incorporation of Histamine into Liver Protein *in vivo*

Recently we have described an enzyme system which catalyses the exchange of the amide group of protein-bound glutamine with primary amines, such as methylamine, putrescine, histamine, serotonin, etc., and also hydroxylamine and ammonia[1]. The livers of rodents, and in particular of guinea pigs, are potent sources of the enzyme. This calcium-dependent enzyme not only catalyses the exchange with primary amines but also the hydrolysis of the amide group of protein-bound glutamine[2]. In the absence of added amine the ε-amino group of protein-bound lysine may act as the replacing agent[3]. The enzyme was named 'transglutaminase'. All mammalian organs examined, and in particular liver and brain, contain proteins which may act *in vitro* as amine-accepting substrates, as are also a number of purified proteins, such as casein, lactoglobulin, insulin, pepsin, etc.

Whereas there is no difficulty in demonstrating transglutaminase action *in vitro*, in early experiments attempts to show fixation of administered amines in the liver proteins of mice were unsuccessful even after experimental liver damage (carbon tetrachloride) or after nephrectomy. The possibility was considered that proteins modified by incorporated amines may have antigenic properties and could be involved in auto-immune reactions. Since *H. pertussis* vaccine enhances anaphylactic sensitization its effect and that of a lipopolysaccharide from *S. typhosa* on liver transglutaminase[4] and histamine incorporation into liver proteins was studied. In mice, 16 hr. after a single injection of lipopolysaccharide, the transglutaminase-level in liver was increased up to three-fold. The enzyme was assayed in the 100,000*g* supernatant fluid of 0·25 molar sucrose liver homogenates with a tryptic digest of casein as substrate and hydroxylamine as replacing amine[2]. *H. pertussis*

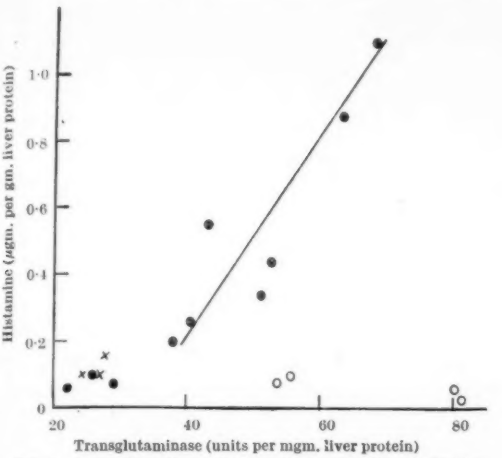

Fig. 1. Transglutamine activity and concentration of protein-bound histamine in mouse livers. Transglutaminase unit = optical density (525 mμ) × 1,000 of the Fe^{+++} complex of hydroxamic acid formed after 10 min. incubation at 37° C. Normal mice (●); normal mice +37·5 mgm./kgm. of histamine (×); mice treated with 5 mgm./kgm. of *S. typhosa* lipopolysaccharide (○); mice treated with lipopolysaccharide, and 37·5 mgm./kgm. of histamine (⊗)

vaccine acted similarly, but was less effective in raising the enzyme-level. Histamine (37·5 mgm./kgm.) was administered intraperitoneally to groups (6–10) of normal and lipopolysaccharide-treated mice which were killed 30 min. later. Small portions of each liver were homogenized together for enzyme determination; the pooled remainder of the livers was precipitated by trichloracetic acid, the protein purified and traces of adsorbed histamine removed by thioglycollate treatment. Histamine was extracted from the ammonia-freed acid hydrolysates of the purified liver proteins using a modification of the method described by Shore *et al.*[5] and assayed on the superfused guinea pig ileum. The results of enzyme and histamine determinations are shown in Fig. 1. The histamine content of the liver proteins from normal or lipopolysaccharide-treated mice with or without histamine injection was less than could be detected and the points shown indicate the limits of the sensitivity of the assay. The liver proteins of lipopolysaccharide-treated animals after histamine injection contain significant amounts of histamine, the quantity of which correlated with the transglutaminase-level ($r = 0{\cdot}9$; $P < 0{\cdot}001$). The validity of the above results was further confirmed in experiments in which histamine labelled with carbon-14 was administered.

It should be emphasized that the increase of enzyme activity and protein-bound histamine does not necessarily represent a causal relationship, nor do the results permit the conclusion that the histamine is incorporated by exchange with the amide group of glutamine bound in liver protein. In order to demonstrate increased histamine fixation in liver protein of lipopolysaccharide-treated mice, it was necessary to administer large doses of histamine. This raises the question whether histamine is the natural replacing amine and experiments are now in progress to study the conditions of *in vivo* incorporation of other amines. Experiments testing the antigenic properties of proteins into which histamine was incorporated *in vitro* by transglutaminase have up to now given equivocal results.

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ISABEL WAJDA
MICHAEL GINSBURG*
HEINRICH WAELSCH

New York Psychiatric Institute
and College of Physicians and
Surgeons,
Columbia University,
New York.

* Permanent address, Department of Pharmacology, University of Bristol.

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Comparison of Short-Circuit Current and Net Water Movement in Single Perfused Proximal Tubules of Rat Kidneys

THE major portion of renal salt and water reabsorption occurs within the proximal convoluted tubule, amounting in mammalian kidneys to about 80 per cent of the quantities present in the glomerular filtrate. Based on indirect evidence, proximal sodium transport has been postulated to be active. Recently, however, results obtained in stop-flow experiments were interpreted in such a way as to indicate passive bulk movement of tubular fluid as the predominant mode of proximal tubular salt transfer. Micropuncture work on rats has demonstrated unequivocally the existence of active sodium transport across proximal epithelium[1], but did not provide information as to the relative contribution of active sodium transport to the overall net movement of this ion species. The present work represents an attempt to clarify this point by correlating active transport of ions as measured by the short-circuit method with measurements of net movement of water under comparable conditions. Assuming that proximal fluid reabsorption is isosmotic—an assumption in agreement with all known measurements of osmotic pressure of proximal tubular fluid—net water movement can be used as an index of net transport of sodium. Thus short-circuit current can be correlated with net movement of sodium.

Experiments were performed on rat kidneys *in vivo*. Short-circuit current was measured by means of the original short-circuit method as described by Ussing and Zerahn[2], using double-barrelled electrodes[3], or by a modification as described by Karger *et al.*[4]. By definition, short-circuit conditions occur when the trans-membrane potential difference is reduced to zero. Under such conditions the current generated by the epithelium passes through

an outer circuit of zero effective resistance. Applied to the renal tubule, the short-circuit current is that current which, applied from an external battery, renders the electrical resistance of a probing micro-electrode within the tubular lumen identical to that of the same electrode when it is outside the lumen. Thus the resistance of the membrane is effectively reduced to zero. Since electrolyte concentrations are nearly identical in proximal tubular fluid and interstitial fluid, the current measured under these conditions must be generated by active ion transport.

All measurements of short-circuit current were performed on tubular fluid segments one diameter in length, isolated from the rest of the tubular fluid by means of columns of mineral oil. The reason for this choice of distance between the bordering oil droplets arises from conclusions derived by cable analysis. In this type of analysis the renal tubule is treated as an electrically leaky cable, and a quantitative measure of its resistance to passive ion flow can be obtained. Use was made of equations derived by Hodgkin and Rushton for the giant axon of the squid[5]. Assessment of the length constant was made possible by passing a known current through one micro-electrode into the tubular lumen and recording voltage displacements at known varying distances within the same tubule by means of a second micro-electrode. With anodic stimulating currents of 10^{-6} amp., voltage amounted to 14 mV. (2 observations, range 8–17 mV.) at 15μ, 9·6 mV. (6 obs., range 6–14 mV.) at 30μ, 5 mV. (2 obs., range 4–6 mV.) at 60μ, and 3·3 mV. (2 obs., range 2·5–4·0 mV.) at an interelectrode distance of 90μ. These results indicate that a current applied through the stimulating micro-electrode leads to a decline in voltage amounting to 63 per cent of its original value at a distance of about 55μ. An identical decline of current intensity can be predicted provided that the tubular epithelium behaves as an ohmic resistance. Therefore, experiments were performed in which the voltage-current relationship was directly examined. With varying anodic and cathodic d.c. currents (up to 3×10^{-6} amp.), proportional displacements of voltage were recorded. Thus proximal tubular epithelium behaves as a linear, non-rectifying circuit element, and the decline of a locally applied current can be predicted from the value of 55μ obtained for the length constant. From these findings it can be concluded that a distance of one tubular diameter (30μ) between the isolating oil droplets is adequate to assure constancy of current density and effective short-circuiting. The average short-circuit current, as measured in a series of 10 experiments by means of the method of Karger *et al.*[4], amounted to 100×10^{-10} amp./$2{\cdot}8 \times 10^{-5}$ cm.^{2} (*S.E.* $\pm$ 20 per cent). Using the approach of Ussing and Zerahn[2], in which double-barrelled electrodes were applied, the average short-circuit current obtained in 6 experiments amounted to 101×10^{-10} amp./$2{\cdot}8 \times 10^{-5}$ cm.^{2} (*S.E.* $\pm$ 7 per cent). The pooled data of these two series of short-circuit current measurements correspond to a net ion transport of 34×10^{-10} equiv./cm.^{2}/sec.

The measurements of short-circuit current were compared with the net movement of sodium ions as calculated from water movement observed in perfusion experiments on single proximal tubules of the rat kidney. In this technique coloured mineral oil is injected into the tubular lumen and split by a small amount of perfusion fluid. The position of the isolated column of perfusion fluid is then secured by injecting mineral oil through a second pipette into the most distal surface loop of the proximal tubule. The perfusion fluid is left within the lumen for a short period, usually about 30 sec., and is then collected through the second, distally located pipette, while mineral oil is injected through the proximal micro-pipette.

Tubules were perfused with fluid containing 145, 100, and 10 m.equiv./l. of sodium. All perfusion fluids were kept isosmotic to plasma by addition of adequate amounts of mannitol. ^{14}C-inulin concentration ratios of collected and injected perfusion fluid were taken as an index of net water movement. At an intratubular sodium concentration of 145 m.equiv./l., water movement amounted to an average value of 59 per cent of the injected volume (4 obs., range 50–67 per cent), at 100 m.equiv./l., to 31 per cent (2 obs., 30 and 31 per cent), whereas at 10 m.equiv./l. the direction of net water movement was reversed and intratubular fluid volume increased by 46 per cent (3 obs., range 41–50 per cent). It should be noted that water reabsorption was reduced to a mean of 5 per cent of the injected volume (4 obs., range -6 to $+17$ per cent) when sodium cyanide at a concentration of 4×10^{-3} *M*/l. was added to the perfusion fluid containing 145 m.equiv./l. of sodium. It may be concluded from these results that a decrease in intratubular sodium concentration results in a decrease in water reabsorption. However, the net water movement may not be ascribed solely to the effect of the intratubular concentration of sodium chloride, since mannitol also was present in the perfusion fluid. To correct the above results for water movement secondary to mannitol flux, a second series of 9 experiments was performed under the same experimental conditions, except that ^{14}C-mannitol was measured rather than ^{14}C-inulin. The ratios of concentrations of ^{14}C-mannitol between collected perfusate and injected perfusion fluid averaged 0·31 (4 obs., range 0·17–0·49) at 10 m.equiv./l. intratubular sodium, and 0·84 (5 obs., range 0·63–0·94) with perfusion fluid containing sodium at a concentration of 100 m.equiv./l. Calculation of the net water movement which may be ascribed to transport of sodium chloride, after correction for mannitol-induced transfer[6], shows that net movement of sodium chloride out of the tubule can take place against a considerable concentration gradient. Water movement ceases between a solution containing 88 m.equiv./l. sodium and one containing sodium at a concentration of 145 m.equiv./l. When the concentration gradient exceeds 57 m.equiv./l., the direction of water movement, ascribed to sodium transfer, is reversed and water enters the tubule. The magnitude of this critical gradient is somewhat greater than that found during free flow conditions in micropuncture studies on rats during osmotic diuresis[1].

Net movement of water observed in experiments in which tubules were perfused with fluid containing 145 m.equiv./l. of sodium could be expressed as net movement of sodium without correction for mannitol flux. In this calculation a mean tubular radius of 15μ was used in estimating the exposed tubular surface area. Furthermore, the assumption was made that proximal fluid absorption is isosmotic and that tubular fluid volume changes as a linear function of time. The calculated mean trans-tubular net flux of sodium, thus obtained, amounts to 30×10^{-10} equiv./cm.^{2}/sec.

The results obtained in this work indicate that the critical sodium concentration gradient at which

net movement of sodium across proximal tubular epithelium stops is 57 m.equiv./l. Comparison of measurements of short-circuit current and calculated sodium reabsorption obtained from data on perfusion experiments indicates that the major portion, possibly all, the proximal sodium reabsorption in the rat kidney is active in nature.

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ERICH E. WINDHAGER*
GERHARD GIEBISCH†

Department of Physiology,
Cornell University Medical College,
New York City.

* Supported in part by a grant from the John Polachek Foundation.
† Established Investigator of the American Heart Association.

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CYTOLOGY

Cytology of Some Genera of Polypodiaceae in Eastern India

POLYPODIACEAE (*sensu* Copeland[1]), a part of the once comprehensive family Polypodiaceae (*sensu lato*), comprises 65 genera, occurring mostly as epiphytes in the tropical and subtropical evergreen and semi-evergreen forests. The genera are characterized by simple, pinnatifid or rarely pinnate fronds with round and rarely linear or acrostichoid typically exindusiate sori. A thorough analysis of the literature together with the study of herbarium specimens available in the Central National Herbarium, Sibpore, West Bengal, and Eastern Regional Herbarium of the Botanical Survey of India, Shillong, Assam, suggests that the family is represented in India by 26 genera and 104 species[2], of which 91 species are distributed in various parts of eastern India, which incidentally appears to be richest in the fern flora in the whole country.

Although Manton[3] has worked out the cytology of 40 species, mostly from Ceylon and Malaya, none of the Indian plants belonging to the family Polypodiaceae (*sensu* Copeland, 1947) has yet been studied cytologically (the first report of the basic number in *Paraleptochilus decurrens* (Bl.) Copel. from Darjeeling has been published only recently by S. S. Bir in *The Nucleus*, **3**, 121; 1960) despite much work on the cytotaxonomy of the other families of ferns. As a part of the larger scheme of undertaking a cytotaxonomic revision of the predominantly epiphytic family Polypodiaceae, the East Indian species are being investigated. The present communication records the chromosome numbers unequivocally established for the species listed in Table 1.

While the present findings confirm the basic chromosome numbers for the genera *Lepisorus*, *Pyrrosia*, *Microsorium*, and *Crypsinus* reported earlier by Manton and Sledge[3], new records of basic chromosome numbers for four genera are now established, namely, *Arthromeris* with $n = 37$ (Fig. 1), *Colysis* with $n = 36$ (Fig. 2), *Loxogramme* with $x = n = 35$ (Figs. 3 and 4), and *Pseudodrynaria* with $n = 36$ (Fig. 5). Although Manton reported three different basic numbers, namely, $x = 35$, 36 and 37, in three species of *Lepisorus* from Ceylon, two species studied by us from eastern India each show $n = 35$ only, whereas two other species show the lowest basic chromosome numbers, namely, $x = 11$ and 13, discovered so far in the family. While the genus *Pyrrosia* appears to be characterized by $n = 37$,

Table 1

1.	*Arthromeris wallichiana* (Sprg.) Ching	Shillong	$n = 37$	Diploid
2.	*Colysis elliptica* (Thbg.) Ching var. *pothifolia* (Don) Ching	Cherrapunji	$n = 36$	,,
3.	*Crypsinus crenatopinnatus* (Clarke) Copel	Shillong	$n = 36$	,,
4.	*Crypsinus oxylobus* (Wallex-Kunze) Sledge	,,	$n = 35$	,,
5.	*Lepisorus excavatus* (Bory) Ching	,,	$n = 35$	,,
6.	*Loxogramme avenia* (Bl.) Pr.	Jowai	$n = 35$	,,
7.	*Loxogramme involuta* (Don) Pr.	Shillong	$n = 70$	Tetraploid
8.	*Loxogramme lanceolata* (Sw.) Pr.	,,	$n = 35$	Diploid
9.	*Loxogramme scolopendrina* (Bory) Pr.	,,	$n = 35$	,,
10.	*Microsorium membranaceum* (Don) Ching	Cherrapunji and Pynursla	$n = 36$	,,
11.	*Microsorium pteropus* (Bl.) Copel.	Cherrapunji	$n = 36$	,,
12.	*Microsorium superficiale* (Bl.) Ching	Shillong	$n = 36$	,,
13.	*Pseudodrynaria coronans* (Wall.) Ching	Kameng F. D.	$n = 36$	,,
14.	*Pyrrosia flocculosa* (Don) Ching	Jowai	$n = 36$	,,
15.	*Pyrrosia mannii* (Gies.) Ching	Shillong	$n = 37$	,,
16.	*Pyrrosia subfurfuracea* (Hk.) Ching	Assam	$n = 37$	,,
17.	*Lepisorus pseudonudus* Ching	,,	$2n = 39$	Autotriploid
18.	*Lepisorus* sp. nov.	Jowai	$n = 22$	Diploid

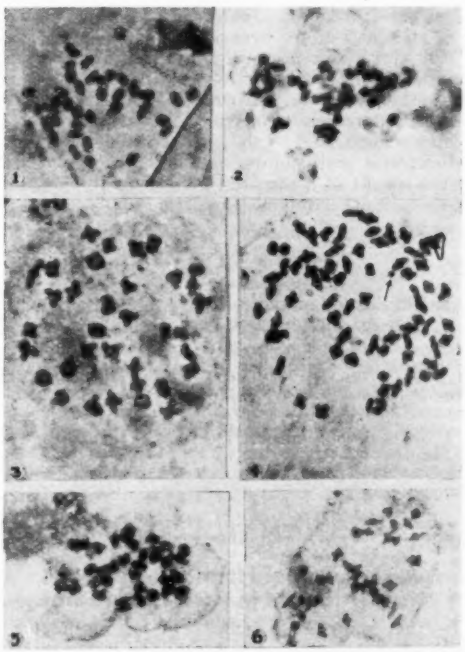

Fig. 1. Samples of meiotic behaviour in wild plants of Polypodiaceae of eastern India (all × 575) from permanent acetocarmine squashes prepared by S. N. Patnaik and photographed by C. C. Mukerjee: (1) *Arthromeris wallichiana*, a diploid ($n = 37$); (2) *Colysis elliptica* var. *pothifolia*, a diploid ($n = 36$); (3) *Loxogramme lanceolata*, a diploid ($n = 35$); (4) *Loxogramme involuta*, a tetraploid ($n = 70$, arrow indicating an extremely small bivalent); (5) *Pseudodrynaria coronans*, a diploid ($n = 36$); (6) *Pyrrosia flocculosa*, a diploid ($n = 36$)

Pyrrosia flocculosa alone shows a deviation with $n = 36$ (Fig. 6). The finding of $n = 36$ in *Pseudodrynaria coronans* (Wall.) Ching as distinct from $n = 37$ in three other species of *Drynaria* studied (Manton and Sledge[3]) lends support to the separation of *Drynaria coronans* (Wall.) J. Sm. for the construction of the monotypic genus *Pseudodrynaria* C. Chr. Of the four species of *Loxogramme* cytologically investigated, three species are diploids each with $n = 35$, whereas *L. involuta* is a tetraploid species showing unmistakably $n = 70$, with a single conspicuously small bivalent at diakinesis. Thus *Loxogramme* is characterized by the basic number $x = 35$ and not 36 (ref. 4). Hence, Manton's report of the chromosome number of *L. involuta* from Ceylon as $n = 70$ to 72 at meiosis and as "probably of the order of 140 in root tip" and her suspicion of the basic number as "probably 36" need revision in view of the unequivocal finding of $x = 35$ in four species of *Loxogramme* in eastern India. Manton (personal communication, 1961) agrees with this finding on the basis of her recent studies of *L. avenia* from Asian tropics.

Copeland's suggestion for merging *Arthromeris* with its parent genus *Crypsinus* to form a natural group is discounted by our discovery of $n = 37$ in *A. wallichiana* from Shillong as against $n = 36, 35, 33$ in *Crypsinus* reported from Eastern India, Ceylon and Malaya. On the other hand, the cytological findings of discordant basic numbers in *Crypsinus* corroborate Copeland's observations regarding the unnaturalness of the genus.

The discovery of the same basic chromosome number, namely, $n = 36$, in the genera *Microsorium*, *Colysis*, and *Leptochilus*, shows close affinity between them and may afford cytological evidence in favour of Holttum's contention of the origin of *Leptochilus* from *Microsorium* through *Colysis*-like ancestors.

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G. Panigrahi
S. N. Patnaik

Botanical Survey of India,
Eastern Circle,
Shillong.

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HÆMATOLOGY

Association of Hæmoglobin *N* and Microcythæmia in a Sardinian Family

In the course of a survey of the frequency of microcythæmic syndromes conducted in the summer of 1960 in Sardinia[1], we encountered a family in which were present both an abnormal hæmoglobin, faster than Hb *A*, and the microcythæmic (or thalassæmic) character.

The father (Table 1) was found to be a carrier of abnormal hæmoglobin; the mother was a carrier of microcythæmia; the first son, Gaet., was completely normal; the other two sons, Aless. and Franc., twins, were those that brought the family under our observation, because it was thought that they were affected by Cooley's disease. Indeed, both showed paleness of the skin and of the mucous membranes, skull slightly increased in volume, spleen enlarged to three finger-breadths below the costal margin, and liver palpable 2 cm. below the right costal margin.

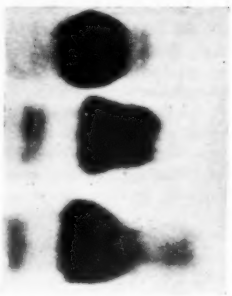

Fig. 1. Paper electrophoresis of Hb Bart's in cord blood (above), Hb *N* (at centre) and Hb *H* (below). Glycine buffer *p*H 8·8, ionic strength 0·04. Staining with bromophenol blue. Mobility of Hb *N* is similar with that of Hb Bart's; but less than that of Hb *H* *in toto*

It has not been possible to conduct a hæmatological examination of Aless.: the blood smears, however, showed very intense erythrocytic alterations, quite similar to those of Cooley's disease. The other twin brother, Franc., presented an evident anæmia with hæmatological characters of microcythæmia (hypochromia, microcytosis, diminished cell fragility) and intense alterations of the morphology. Furthermore, both twins were carriers of fast hæmoglobin, while the proportion of Hb A_2 was less than the normal. The abnormal hæmoglobin presented the following characters: (1) on paper and starch electrophoresis at alkaline *p*H (Fig. 1) the hæmoglobin had a mobility identical with that of Hb Bart's, but lower than that of Hb *H* *in toto*; (2) on electrophoresis at acid *p*H (Fig. 2), the abnormal hæmoglobin tends to separate from Hb *A*, forming a thicker hæmoglobinic spot; (3) on agar-gel electrophoresis at acid *p*H, this abnormal hæmoglobin cannot be distinguished from Hb *A*; (4) on 'Amberlite *IRC*-50' chromatography at acid *p*H, the abnormal hæmoglobin precedes Hb *A* and separates from it (Fig. 3), but it is slower than Hb *F*.

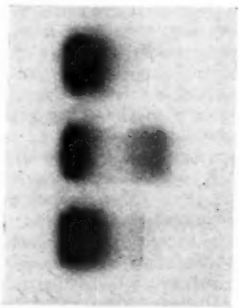

Fig. 2. Starch-gel electrophoresis of Hb *N* (above and below) and Hb *H* (centre). Phosphate buffer *p*H 6, ionic strength 0·12. Staining with amidoschwarz. The Hb *N* tends to separate from Hb *A* and forms a thicker spot than Hb *H*

Table 1. HÆMATOLOGICAL AND HÆMOGLOBINIC FINDINGS

Name and age (yr.)	Hb (gm./100 ml.)	Red blood cells (millions per c.mm.)	Mean corpuscular volume (μ^3)	Mean concentration of corpuscular hæmoglobin (per cent)	Hæmolysis in 0·4 sol. of Simmel's method	Blood film	Hæmoglobin types*			Percentage alkali-resistant Hb of total Hb
							Hb A_2	Hb A_1	Hb N	
Father, 36	15·2	4·7	95	33·7	97·9	Normal	1·35	62·2	36·4	1·06
Mother, 39	11·5	6·0	65	29·3	88·4	Anisocytosis, poikilocytosis, target cells, oval cells, moderate hypochromia	4·58	95·4	abs.	2·05
Gaet., 4	13·2	4·2	91	34·3	97·7	Normal	2·61	97·4	abs.	1·25
Aless., 1½						Marked anisocytosis and poikilocytosis, numerous target cells, oval cells and microcytes, marked hypochromia	1·14	62·0	36·9	1·98
Franc., 1½	6·8	3·6	67	27·8	94·5	Marked anisocytosis, poikilocytosis, microcytosis, numerous target cells and oval cells, marked hypochromia	0·78	63·5	35·7	2·36

* Percentages by starch-block electrophoresis, according to Kunkel *et al.*

In all the subjects (Table 1), the proportion of fast hæmoglobin was about 36 per cent, and that of Hb *a-r* was normal. In the ultra-violet region, the fast fraction isolated has a normal absorption spectrum; the resistance to alkali of this fraction is normal.

It is evident that the above characters correspond to those of Hb Liberia I of Robinson *et al.*[2] or Hb *N* of Ager and Lehmann[3]. Therefore, the present hæmoglobin may be classified as Hb *N*. The behaviour of Hb *N* on agar-gel electrophoresis at acid *p*H is not different from that of Hb *A*.

The present observation is, so far as we know, the first of Hb *N* identified in the Italian population. Association with the microcythæmic trait is not surprising, as the family is Sardinian and microcythæmia has an average frequency of 23 per cent on the Island[1]. It is interesting to visualize the presence of an anæmic-splenomegalic syndrome in the twin brothers, who are carriers of abnormal hæmoglobin. True Cooley's disease cannot be involved, since the microcythæmia is absent in the father, and illegitimacy is ruled out by the presence of the abnormal hæmoglobin in the father and sons. However, it is quite probable that both sons are carriers of the microcythæmia inherited from the mother, and that their illness is due to the association of microcythæmia and Hb *N*. Therefore, this would be another example of illness due to the association of two different hæmatological anomalies, the first example of which is the microdrepanocytic disease which we have identified[4,5].

The proportion of Hb A_2, which is strangely low in the twins, suggests the consideration that the presence of an abnormal hæmoglobin, faster than Hb *A*, may represent the determining factor for the reduced proportion of Hb A_2, as we see also in the carriers of Hb *H*.

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E. SILVESTRONI
I. BIANCO

Istituto d'Igiene, Università di Roma,
Centro di Studi della Microcitemia
e delle Anemie Microcitemiche,
Roma.

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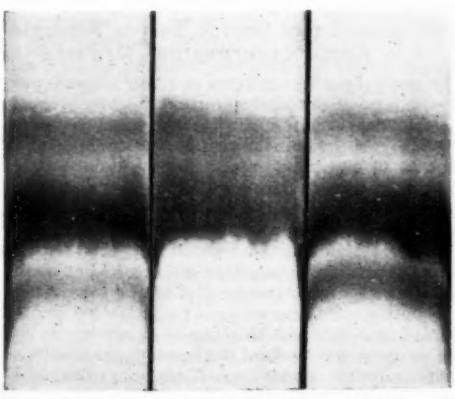

Fig. 3. Chromatography on columns of 'Amberlite *IRC*-50' of Hb *N* (left and right) and normal adult Hb (centre). Citrate buffer *p*H 6, ionic strength 0·05. Hb *N* precedes Hb *A* and separates clearly from it

Anti-Leucocyte Cytoplasm, Anti-Leucocyte Nuclei and Anti-Platelets in Disseminated Lupus Erythematosis

THE indirect antiglobulin consumption test[1,2] has been carried out on human leucocyte cytoplasm, leucocyte nuclei and platelets, using sera containing anti-leucocyte and/or platelet antibodies from non-transfused or minimally transfused patients: six disseminated lupus erythematosis (DLE), five idiopathic leuco-thrombopenias (ILT), four idiopathic thrombocytopenic purpuras (ITP). The auto-antibody nature of these substances was confirmed by the positive direct antiglobulin consumption test performed on the patients' own leucocytes and/or platelets. Three sera containing iso-leucocyte and platelet antibodies from three multiple transfused patients were also tested.

Leucocyte nuclei were isolated from liquefied cytoplasm[3]. Cytoplasmic proteins were obtained from the supernatant by ethanol precipitation (Colombani, J., unpublished work). Four absorptions were performed systematically and total absorption was always controlled. Elution was performed by heating the sensitized deposit for 30 min. at 56° C. in hypertonic saline. Sera, before and after absorption, and eluates were respectively tested against the three antigens (Table 1).

Table 1. Anti-cytoplasm: Anti-Nucleus and Anti-Platelet Antibodies studied by the Indirect Antiglobulin Consumption Test

		Transfusions	Pregnancies	Direct antiglobulin consumption test L	P	Before absorption* N	C	P	After absorption on leucocyte nucleus* Absorbate N	C	P	Eluate N	C	P	After absorption on leucocyte cytoplasm* Absorbate N	C	P	Eluate N	C	P	After absorption on platelet* Absorbate N	C	P	Eluate N	C	P
DLE	ALI.	0	0	+	+	8	7	8	0	6	8	3	1	0	7	0	8	0	4	0	8	7	0	0	0	3
	PAG.	1	0	+	+	7	6	8	0	6	8	2	0	0	6	0	8	0	4	0	5	6	0	0	0	8
	SOT.	3	4	−	(+)	6	6	8	0	5	8	3	0	0	7	0	8	0	3	0	7	5	0	0	0	3
	BAS.	0	0	+	+	4	6	7	0	6	7	1	0	0	5	0	7	0	3	1	5	5	0	0	0	2
	LAP.	0	0	+	+	5	5	7	0	4	6	1	0	0	5	0	7	0	2	0	5	4	0	0	0	3
	HUB.	0	1	+	+	8	8	8	0	8	8	1	1	0	7	0	8	0	5	1	8	8	0	0	0	3
ILT	BUR.	0	0	+	+	0	8	7	0	8	3	0	0	0	0	0	8	0	4	0	0	8	0	0	0	3
	D'AM.	0	2	+	+	0	6	5	0	6	4	0	0	0	0	0	5	0	3	0	0	6	0	0	0	2
	CHA.	1	1	+	+	0	8	7	0	7	6	0	0	0	0	0	6	0	4	0	0	7	0	0	0	3
	DEY.	3	1	+	+	0	8	8	0	8	8	0	0	0	0	0	8	0	6	0	0	8	0	0	0	3
	FAU.	0	4	+	+	0	5	4	0	5	4	0	0	0	0	0	4	1	4	0	0	5	0	0	0	1
ITP	CAR.	0	4	−	+	0	0	6	0	0	5	0	0	0	0	1	5	0	0	0	1	1	0	0	0	3
	CARL.	2	2	−	+	0	0	5	0	1	4	0	0	0	0	1	4	0	0	0	0	0	0	0	0	2
	MAL.	2	1	−	+	0	0	6	0	0	6	0	0	0	0	0	5	0	0	1	0	0	0	0	0	2
	MAC.	0	0			0	0	5	0	0	6	0	0	0	0	0	6	0	0	0	0	0	0	0	0	1
Iso-antibodies	CARP.	122	3	−	−	0	8	8	0	8	8	0	0	0	0	0	8	0	4	0	0	6	0	0	0	4
	RIC.	147	1	−	−	0	7	6	0	5	5	0	0	0	0	0	6	0	4	0	0	6	0	0	0	3
	ROG.	27	2	−	−	0	7	6	0	6	5	0	0	0	0	0	5	0	3	0	0	6	0	0	0	3

L, Leucocytes; *C*, leucocyte cytoplasm; *N*, leucocyte nucleus; *P*, platelet.
* The figures are the number of geometric dilution lost by the antiglobulin titre (titrated against *O* Rh + RBC sensitized by incomplete anti-*D*) after contact with the antigens sensitized by the auto- or iso-antibody.

DLE sera contained three distinct antibodies against cytoplasm, nuclei and platelets. ILT sera contained two antibodies against cytoplasm and platelets. ITP sera contained only one antibody against platelets. The sera of patients with multiple transfusions behave like ILT sera.

Table 2. Differentiation between the Anti-Leucocyte and Anti-Platelet Antibodies detected by the Indirect Antiglobulin Consumption Test

	Action on animal leucocyte or platelet	Agglutinating power on leucocyte and/or platelet	Antibodies not detected by some antiglobulin sera
Anti-leucocyte antibodies			
DLE	+ +	±	−
ILT	−	−	−
Iso-antibody	−	+ +	−
Anti-platelet			
DLE	+ +	±	−
ILT	−	−	−
ITP	−	−	+ +
Iso-antibody	−	+ +	−

The three anti-cytoplasm antibodies are not identical. DLE anti-cytoplasm reacts with the leucocyte cytoplasm of animal origin (ox, sheep, pig, horse), though the others do not react. ILT sera are not leuco-agglutinating, though iso-leuco-antibodies used in this work are strongly agglutinating.

The four anti-platelet antibodies are not identical. DLE anti-platelet reacts with animal platelets, though the others do not. ILT and ITP sera are not thrombo-agglutinating, though iso-thrombo-antibodies used in this work are agglutinating.

Table 3. Antiglobulin Consumption Test on Leucocyte Nucleus and Cytoplasm and on Platelets from Various Animal Species

Antibodies from	Name	Ox N	C	P	Sheep N	C	P	Pig N	C	P	Horse N	C	P
DLE	ALI.	8	7	3	5	7	3	6	7	6	6	6	5
	BAS.	7	6	2	7	6	2	8	6	4	6	5	5
	SOT.	6	7	2	8	6	2	7	5	3	6	7	5
	HUB.	7	5	2	6	7	3	7	4	4	6	6	4
ITP	CARL.	0	0	0	0	0	0	0	0	0	0	0	0
	CART.	0	0	0	0	0	0	0	0	0	0	0	0
ILT	BUR.	0	0	0	0	0	0	0	0	0	0	0	0
	DAM.	0	0	0	0	0	0	0	0	0	0	0	0
Iso-antibodies	CARP.	0	0	0	0	0	0	0	0	0	0	0	0
	RIC.	0	0	0	0	0	0	0	0	0	0	0	0
Control		0	0	0	0	0	0	0	0	0	0	0	0

ITP antiplatelet antibodies react with a component of the antiglobulin sera which is different from the component reacting with the three other anti-platelet antibodies[4] (Tables 2 and 3).

These findings show that DLE sera possess an anti-cytoplasm factor at least as strong as the anti-nucleus factor when using the antiglobulin consumption test, although with fluorescent antiglobulin only the nucleus seems to be sensitized. Anti-cytoplasm antibodies in DLE were already suspected[5,6] but had not been directly demonstrated in human material.

J. Dausset
J. Colombani
M. Colombani

Centre National de Transfusion Sanguine,
Paris, XV^e.

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IMMUNOLOGY

Elimination of Bovine Serum Albumin in Acute Experimental Uræmia

In patients suffering from acute or chronic uræmia the production of classical antibodies has been shown to be within normal limits[1-4]. The Mantoux test may be positive in chronically uræmic humans, implying that the formation of certain intra-cellular antibodies is also maintained. However, skin homografts survive for prolonged periods in the uræmic human[1,2], indicating that the onset of transplantation immunity is delayed. Medawar[5] has stated that the instruments of transplantation immunity are antibodies bound to cells of the lymphoid series and not serum antibodies. Skin homografts also survive for extended periods in uræmic rabbits[6], and this has been shown to be due to a delay in the onset of the immune responses in the draining lymph nodes (ref. 6 and unpublished work by Barwell, R. G., *et al.*). Having demonstrated the effect of uræmia on the production of the cell-bound antibodies of transplantation immunity, we decided to investigate (*a*) the elimination of a hetero-

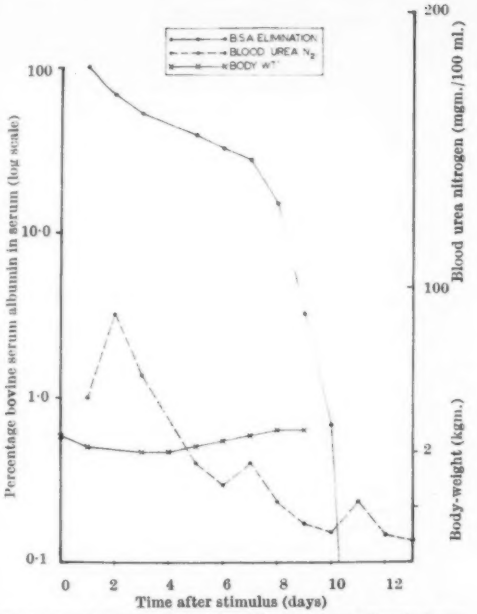

Fig. 1. Graph to show elimination of bovine serum albumin, levels of blood urea nitrogen and body-weight in a mildly uræmic rabbit

logous serum protein and (*b*) the production of serum antibodies in the uræmic rabbit.

Rabbits of Flemish giant strain each weighing 2·5–3·5 kgm. were used. The animals were anæsthetized by intravenous sodium pentobarbitone. The left kidney was removed from each animal and the right renal vascular pedicle occluded for 1 hr. by a non-crushing arterial clamp. After release of the clamp, the kidney was seen to flush and was then replaced within the abdominal cavity and the wound closed. Immediately after removal of the clamp, each rabbit received 5 ml. of a 5 per cent solution of bovine serum albumin (Armour) intravenously. Specimens of blood were taken for immunological examination, and blood urea nitrogen was determined at 24 hr. after the injection of bovine serum albumin and at daily intervals for a further twelve days. The blood urea nitrogen was estimated by the method of Archibald[7] and each animal was weighed daily.

The samples of blood obtained for immunological analysis were allowed to clot, and serum separated by centrifugation and stored in sterile rubber-stoppered glass tubes at 4° C. for at least 7 days to allow precipitation to occur. The sera were then recentrifuged and the bovine serum albumin contents were determined by the constant-antibody precipitation technique. The level of serum bovine serum albumin 24 hr. after injection was taken as 100 per cent, and readings obtained thereafter for each animal were expressed as a percentage of the first reading.

The effect of mild uræmia on the elimination of bovine serum albumin and on the body-weight is shown in Fig. 1. In this animal the blood urea nitrogen rose to 90 mgm. per 100 ml. 48 hr. after operation and then fell, at first rapidly, and then more slowly towards normal levels. Loss of weight occurred but only during the first three days of observation. The elimination of bovine serum albumin in this animal proceeded in a manner which could not be distinguished from normal. After equilibration, a logarithmic phase of elimination occurred lasting four days, followed by a period of rapid 'immune' elimination for the next two or three days before the bovine serum albumin became undetectable. This pattern of response was found in four rabbits.

The results obtained from an animal with severe uræmia are shown in Fig. 2. By the fifth day, the blood urea nitrogen had risen to 190 mgm. per 100 ml. and, after a transient fall, it again rose before death occurred in severe uræmia on the thirteenth post-operative day. A progressive loss of weight occurred during the course of the experiment. After the equilibration phase, the bovine serum albumin was eliminated logarithmically from the blood over the remaining period of observation. Its half-life during the logarithmic phase was 4 days, which is similar to the half-life in the logarithmic phase of normal rabbits. The half-life of endogenous albumin in normal rabbits is 6–8 days; hence despite the absence of the immune phase of elimination in the severely uræmic rabbit, the bovine serum albumin is still recognized as a foreign protein. Two of the three severely uræmic rabbits which showed this pattern of response and survived the experiment had formed serum antibodies when tested on the thirty-fourth post-operative day.

These experiments demonstrate a marked delay in the elimination of bovine serum albumin from the sera of severely uræmic rabbits. It seems likely that the persistence of detectable antigen for longer periods than in the mildly uræmic or normal animal is associated with the impaired production of serum antibodies, but how this occurs is unknown. It has been shown that the elimination of heterologous proteins from the blood stream is also prolonged after

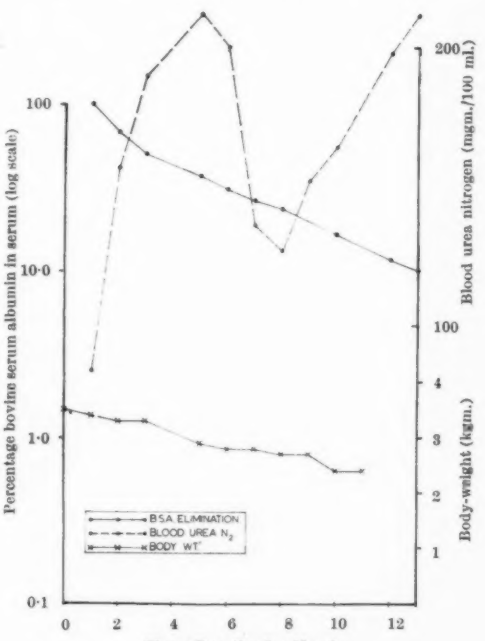

Fig. 2. Graph to show elimination of bovine serum albumin, levels of blood urea nitrogen and body-weight in a severely uræmic rabbit

the administration of the purine metabolic analogue, 6-mercaptopurine[8]. This drug also prolongs the survival of skin homografts in rabbits[10] and renal homotransplants in dogs[9].

A more detailed account of our findings, together with further work on the effect of experimental uræmia on classical immunological responses, will be published later.

G. Gowland*
F. G. Smiddy
R. G. Burwell
F. M. Parsons

Departments of Bacteriology, Surgery, Anatomy and the Medical Research Council Unit on Metabolic Disturbances in Surgery, University of Leeds.

* Present address: Department of Zoology, University College, London, W.C.1.

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Demonstration by Paper Electrophoresis of an Auto-immunological Precipitin possibly due to Fibrinolysins

We have examined recently blood containing fibrinolysins in high titre. The blood was taken from a man who was later diagnosed as suffering from prostatic malignancy. The presence of high-titre fibrinolysins, raised formal acid phosphatase, and greatly reduced fibrinogen content, were all carried out by routine, accepted biochemical methods, and were fully diagnostic. It was then considered that there could be present a circulating auto-immunological factor which we might be able to demonstrate.

The method chosen was what we have called joint paper electrophoresis, and is essentially that of Flynn and Mayo[1], the only addition being that we offer a suitable source of fibrinogen to the serum containing fibrinolysins. We do this by applying plasma from another subject a short distance behind the serum start line, the result being, naturally, two protein separations, one behind the other. Our findings show that it is possible to produce a thin band typical of auto-immunological precipitins. This band appears in the region of the serum γ-globulin separation and we believe it to be an auto-immunological fibrinolysate.

Procedures taken to discover the optimal source of fibrinogen and to qualify our belief have been fully embodied in a paper which we hope to publish in the near future.

Marion I. Cooper
J. R. Sykes

Worthing Hospital,
Worthing, Sussex.

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PATHOLOGY

Immunological Observations in Experimental Silicosis

Evidence which suggests that an immunological mechanism may participate in the pathogenesis of human silicosis has been summarized by Vigliani and Pernis[1]. We have attempted to identify such a mechanism in the experimental disease by applying the fluorescent antibody technique of Coons and Kaplan to rats with silicosis at different stages of development, while observing at the same time the γ-globulin- and conglutinin-levels in their sera.

Silicosis was induced in rats by the intratracheal injection of a quartz suspension under ether anæsthesia, the rats being killed approximately 1–12 months later. Serum was obtained from the rats by cardiac puncture just before death and analysed for protein composition (paper electrophoresis) and for conglutinin titre[2]. Sera were prepared in rabbits against rat whole-serum and against rat γ-globulin obtained by ethanol fractionation[3]. Antibody protein was conjugated with lissamine rhodamine *B* 200[4] and applied to sections fixed in ethanol after preparation from freshly frozen silicotic lung in a cryostat. The customary control procedures were employed.

The serum globulin and conglutinin (Table 1) tended to show changes similar in type to those found by Ceppellini and Pernis[5] in human silicosis. In rats with silicosis more than 6 months the serum globulin was sometimes raised, but there was no elevation in three long survivors. When an increase occurred it mainly affected γ-globulin, though in some animals there was also a slight elevation of β-globulin. Silicotic rats often had a considerable augmentation of conglutinating activity in their sera, but this rise was related neither to the duration of their disease nor to the occurrence of hyperglobulinæmia.

Table 1. Serum Findings in Experimental Silicosis

Duration of disease (months)	Conglutinin titre	Hyperglobulinæmia
1	1 in 32	Absent
1	8	Absent
1	64	Absent
3½	8	Absent
3½	256	Absent
6½	8	Present
6½	128	Present
6½	4	Present
11	64	Present
11	32	Absent
11	128	Absent
12	4	Absent
12	32	Present

By immuno-electrophoresis[6], it was established that the rabbit antiserum to rat whole-serum produced precipitin bands to all components of rat serum proteins, whereas the rabbit antiserum to rat γ-globulin showed the characteristic bands in the appropriate zone with only very slight β-globulin activity. Although many of the rat silicotic nodules showed hyalinization, no fluorescence of their connective tissue was observed in any of the sections treated with conjugated antibody to rat whole-serum or to rat γ-globulin. Some fluorescence did, however, occur within the larger blood vessels and in exudate lining alveoli, and was, presumably, a reflexion of the presence of serum proteins. In view of this negative finding in the fibrous tissue of the nodules it was necessary to provide a positive control to our technique. Sections from a human rheumatoid nodule were therefore treated with a conjugated antiserum to human globulin. Vivid specific fluorescence of the

central hyaline material was obtained, thereby confirming the findings of Vazquez and Dixon[7].

Chemical analysis of the hyalinized connective tissue from human silicotic nodules showed that it consisted of 80 per cent protein, but only about 40 per cent of this was thought to be collagen, the remainder resembling serum β- and γ-globulins[1]. The immunological work of Ceppellini and Pernis[5] supported this view. Using a different technique and experimentally induced silicosis, we have been unable in mature nodules to provide evidence of the presence of globulins similar to those of the serum. Moreover, the conglutinin titre and the occurrence of hyperglobulinæmia bore no consistent relationship to the stage of maturity of the lesions. Our essentially negative results may mean that the induced lesions did not contain globulins or that, if present, our technique was not sufficiently sensitive to detect them. Further chemical and immunological investigations are evidently needed to decide whether or not the pathogenesis of the experimental disease is essentially different from that of human silicosis.

Dr. Nagelschmidt kindly provided the sized quartz dust.

J. H. Jones

Department of Pathology,
University of Wales,
Cardiff.

A. G. Heppleston *

Department of Pathology,
University of Durham.

* Formerly University of Wales.

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Transplantation of Murine Leukæmia by Unconventional Routes

A lymphocytic type of leukæmia which arose spontaneously in a mouse of strain *CBA* has already been described[1]. Investigation of transmission of this leukæmia between mice of the strain of origin revealed the following features: transmission could not be effected by the injection of cell-free homogenates of leukæmic organs into mice either before or after birth; transplantation bioassays of single-cell suspensions of morphologically intact leukæmic cells showed that an average intraperitoneal inoculum of only two cells would consistently achieve successful transplantation to 50 per cent of injected mice (that is, $TD_{50} = 2$ cells); similar very low TD_{50} values were obtained from assays performed by the subcutaneous or intravenous routes; no solid tumours appeared at sites of subcutaneous injection, and no trace of ascites appeared after intraperitoneal injection of the cells.

With the foregoing features of this strain of leukæmia in mind, I was encouraged to attempt transplantation of the cells by several unconventional routes: pervaginally, pernasally, via the conjunctival sac, percutaneously, and by limited surgical incision of the skin using instruments contaminated with viable cells. In each case, the inoculum consisted of a single-cell suspension of viable leukæmia cells in Tyrode solution containing 5 per cent of *CBA* mouse serum; the total number of cells available in each inoculum was within the range 10^4–10^5.

For pervaginal transplantation, a drop of suspension was placed in the vagina with a smooth-tipped Pasteur pipette; pernasal transplantation was effected by placing a drop of suspension on the noses of inverted lightly anæsthetized mice; application to the eyes was made simply by depositing a drop of suspension on the globe of each eye of anæsthetized mice. All the above applications were made with meticulous avoidance of trauma to the mucosal surfaces. For percutaneous transplantation, the fur was plucked from one flank and the skin was scarified with a needle sufficiently lightly to penetrate the keratin layer without drawing blood; a drop of suspension was rubbed into the scarified surface with a glass rod. To test for transplantation of cells from contaminated instruments, scissors which had been dipped in cell suspension were used to remove a small V-shaped segment from the periphery of the auricles of mice, a minor surgical procedure commonly employed to differentiate mice that are to be caged together.

The incidence of successful transplantation by these various routes, together with the survival-times of affected mice following treatment with cells, are given in Table 1.

Table 1. Incidence of Leukæmia, and Survival Time of Affected Mice, following Implantation of Leukæmia Cells at Unusual Sites

Site	Proportion of treated mice developing leukæmia	Survival-times (days)
Vagina	1/4	20
Nasal cavity	11/12	15–17
Conjunctiva	0/22	—
Scarified skin	4/4	24–27
Incised skin	6/6	18–37

It is probable that the success of intravaginal transplantation is influenced by œstrus; an effect of œstrus at the time of painting the cervix with benzopyrene has been demonstrated for the chemical induction of cervical neoplasia[2].

Since most of the cells deposited on the globe of the eye are likely to accompany the tear stream into the naso-lacrymal duct, it may appear surprising that this transplantation route was far less effective than the direct instillation of cells into the nose. On the other hand, the method used to introduce cells into the nose would be expected to result in cells reaching the ethmoid region or being inhaled into the bronchi, where they would come to lie on non-stratified epithelium; cells entering the nasal cavity from the naso-lacrymal duct would be unlikely to reach these regions.

About ten days after application of cells to the scarified skin, temporary enlargement of the regional lymph nodes was observed, suggesting that leukæmia cells may have entered the lymphatics of the skin.

In no case was a local tumour seen at any of the sites of implantation used.

The results of these experiments seem to justify use of the term 'contagion' to describe the means of cellular transplantation reported, and it is clear that a strictly 'aseptic' routine must be followed if mixing of cell strains is to be confidently avoided in laboratories where several different leukæmia strains indigenous to the same mouse strain are maintained by serial transplantation. It is not inconceivable that occasional accidental transplantation could occur from cannibalization of dead leukæmic mice by isologous cage-mates.

XUM

Although a considerably longer latent period usually distinguishes the development of virus-induced leukæmia from that of transplanted leukæmia, the present findings indicate that stringent criteria for cell exclusion must be observed in all experiments which purport to demonstrate subcellular transmission of leukæmia.

All mice dying of leukæmia following the introduction of cells by the unusual routes described showed the typical pattern of generalized visceral infiltration, with no signs referable to the point of entry of the cells. This finding seems to emphasize that evidence for a multifocal origin of spontaneous leukæmia, commonly mooted in text-books of general pathology, cannot be got from histopathological or morbid anatomical study of the leukæmic individual.

Harold B. Hewitt

John Burford Carlill Laboratories,
Westminster School of Medicine,
London, S.W.1.

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BIOLOGY

Effect of Thyroxine Plus Thiourea on the Early Development of the Chum Salmon (*Oncorhynchus keta*)

Available evidence indicates that the effect of the thyroid hormone in fishes is morphogenetic rather than metabolic. Thyroid treatment stimulates growth differentially during development, so that proportions of fins, body-wall, weight and length are altered. Deposition of guanine in the skin of the salmonids[2] and the rate of formation of scales in sturgeons (genus *Acipenser*) are accelerated[3]. Working with the chum salmon, Dales and Hoar[4] found that thiourea slowed down growth and morphogenetic processes. They found that, while the body-wall started growing over the yolk sac very early in the case of alevins treated with thyroxine, this process was considerably retarded in the forms treated with thiourea. The experiment, the results of which are presented here, was undertaken in order to ascertain the effect of a mixture of thyroxine and thiourea on the early development of the chum salmon. It was of interest to find out whether, in a mixture, the effect of thyroxine would be dominant over that of thiourea or vice versa.

Chum salmon eggs were collected by stripping fish migrating into Cultus Lake, B.C., on November 6, 1954. They were fertilized immediately and brought to the University hatchery. The eggs were transferred to 4 troughs (5,000 in each). The capacity of each trough was 10 litres. They were placed in a running tap-water bath; the first trough was the control and contained tap water; the second contained a thyroxine solution (1 : 12,000,000); the third one contained thiourea (0·05 per cent) and the fourth contained a mixture of thyroxine (1 : 12,000,000) and thiourea (0·05 per cent). The solutions were aerated at all times, and their temperatures ranged from 5° to 11° C. during the 92 days of the experiment. Temperature was recorded daily and the solutions changed weekly. Dead eggs were removed once a week.

As a result of heavy mortalities from fungal infection, the experiment was terminated 92 days after it commenced. It is of interest to point out that, while the eggs in the other three tanks suffered heavy fungal attack, none of the eggs in the thiourea tank was infected. Since the eggs in the thyroxine and thiourea mixtures were infected, it may be speculated that the infecting organisms found it possible to thrive in the presence of thyroxine even though thiourea was also present, although they did not survive in a pure thiourea solution.

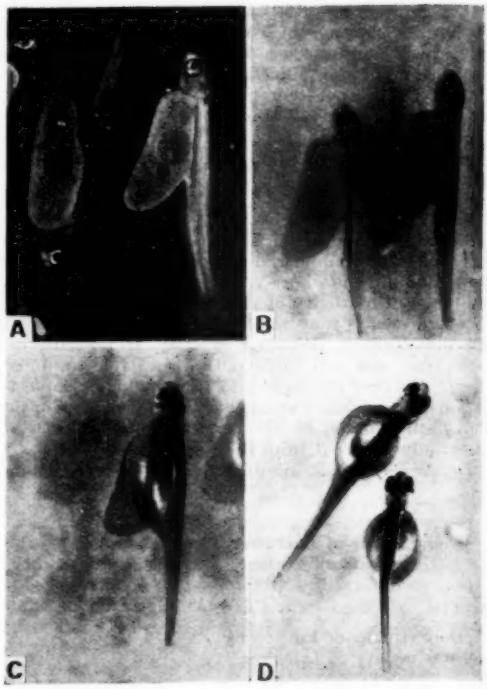

Fig. 1. Alevins soon after hatching. *A*, Control; *B*, treated with thiourea; *C*, treated with thyroxine; *D*, treated with thyroxine + thiourea. Sizes range from 20 to 26 mm. Note the growth of the body-wall and deposition of guanine in *C* and *D*

Eggs in the thyroxine and thiourea mixture hatched first (56 days after fertilization), followed by those treated with thyroxine (60 days), control (64 days) and those treated with thiourea (67 days).

The early development of the chum salmon has already been studied[5]. Development of the embryos prior to hatching, their morphological characteristics before and after hatching, rate of yolk absorption, pigmentation, guanine deposition, rate of heart-beats and mouth and gill movements were investigated. Observations made in the case of control, thyroxine and thiourea groups were similar to those made by Dales and Hoar[4] and will not be repeated here.

Careful examination showed that the development of eggs incubated in a mixture of thyroxine and thiourea solutions is very similar to that of eggs treated with thyroxine alone and the observations of Dales and Hoar[4]. Small variations do occur but are not of any significance. The accompanying photographs (Fig. 1) show the alevins from the four tanks soon after hatching. In these photographs, the similarity between the alevins treated with thyroxine and those treated with thyroxine + thiourea, and the dissimilarity between the alevins treated with the

mixture and those treated with thiourea as well as the control alevins are obvious.

From these results it would appear that during the early development of the chum salmon the influence of thyroxine prevails over that of thiourea. While thiourea exerts some influence in the absence of thyroxine, in a mixed solution the effects of thyroxine are dominant and completely suppress the effect of thiourea, even though the former is present only in a much more dilute form than the latter.

M. A. Ali

Département de Biologie,
Université de Montréal,
Montréal, P.Q.

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Oocyte Numbers as a Diagnostic Character for the Identification of Ammocœte Species

The nature of the relationship between the European brook lamprey *L. planeri* and the closely related parasitic form, *L. fluviatilis*, has been the subject of considerable discussion, especially since the classical studies of Weissenberg[1-3] showed that differences between these species are based on biological rather than morphological criteria. The difficulty of finding taxonomic characters for the identification of their larval stages has been a matter of great inconvenience to workers in this field, especially since the two species often occur together in the same river system.

In view of the great difference in fecundity between the adult brook and river lampreys which produce about 1,000–2,000 and 4,000–40,000 eggs respectively[4-6], the suggestion was made by Weissenberg[3] that there would be corresponding differences in the number of oocytes at the ammocoete stage. This point has now been examined, using ammocoetes from the R. Yeo (Somerset) and R. Nevern (Pembrokeshire), which are known to consist of *L. planeri* only, and others from the Rivers Teifi and Usk, where both species occur.

Although the larval ovary extends almost throughout the entire length of the body cavity, the oocytes tend to occur in greatest number in the region of the mesonephric kidney anterior to the first dorsal fin. Counts have been made of the number of oocytes in random transverse sections of this part of the body for a large number of animals and the results plotted as a frequency histogram (Fig. 1). Since practically all male ammocoetes develop a few oocytes during the earlier stages of larval life, all the groups examined show a fairly high proportion with small oocyte numbers (less than 20). In *planeri* ammocoetes from the R. Yeo only one case has been recorded of more than 45 oocytes per section, but for both the Teifi and the Usk no less than 32 per cent have exceeded this figure, and at the upper limit numbers of more than 100 have occurred. The frequency distributions for the Teifi and Usk also appear to indicate the presence of two groups with smaller and larger oocyte numbers, the latter with a frequency peak at 50–55 oocytes/section. As it seemed probable that the animals with the larger oocyte numbers represented *fluviatilis* ammocoetes, estimates were made of the total numbers of oocytes in the whole ovary in animals selected from the upper and lower ranges of oocyte numbers (Table 1). This was done by the laborious method of taking sample serial sections through the whole ovary at intervals of 1–2 mm. using the microtome to measure these intervals. Measurements of the average oocyte diameters for each animal enable the total number of oocytes to be estimated. For *planeri* ammocoetes (R. Yeo and R. Nevern) these estimates have varied from 4,900 to 10,600 for animals with 25–50 oocytes/section. For material from the Teifi and Usk, ammocoetes with 60 or more oocytes/section have given total counts of 8,300–26,000. Bearing in mind the reported range of egg production in *L. fluviatilis*, it seems safe to refer the higher counts to this form, and, while there may be some overlapping of oocyte numbers in the two species, counts in excess of 14,000 or 65/section may be taken as indicating river lamprey ammocoetes. Although *P. marinus* is known to spawn in the Usk, the possi-

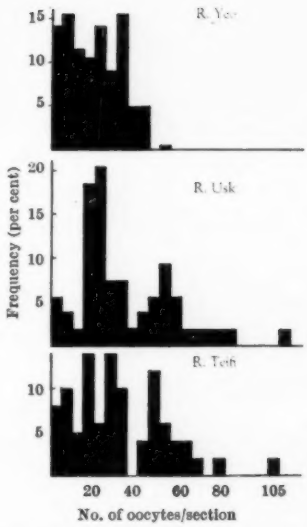

Fig. 1. Frequency histograms for oocyte numbers in ammocoetes from three different rivers

Table 1

	Total length (mm.)	Maximum No. oocytes/section	Total oocytes	Mean oocyte diameter (μ)
River Yeo	53	33	7,200	36
	62	45	8,100	57
	63	50	10,600	47
	68	32	6,100	64
	68	25	5,500	61
	84	27	5,800	75
	125	32	7,400	100
River Nevern	48	29	6,300	48
	48	37	6,500	42
	53	38	9,300	42
	54	28	4,900	38
River Teifi	61	58	11,700	41
	77	110	26,000	40
	78	63	8,300	92
	83	79	15,000	77
	110	35	6,351	103
River Usk	79	108	21,500	59
	83	65	14,600	73
	99	70	18,000	81
	99	81	22,800	70

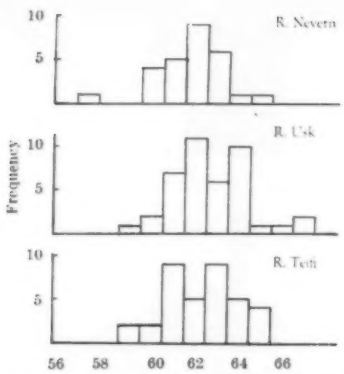

Fig. 2. Frequency distribution of myotome numbers in ammocoetes from three Welsh rivers

bility that ammocoetes with the largest oocyte counts might belong to this species can be excluded, as a number of sea lamprey larvæ which have been examined have given total counts of more than 100,000.

Myotome counts have been made on 104 ammocoetes from the Rivers Nevern, Teifi and Usk and, while the Nevern population gave a unimodal distribution with the maximum frequency at 62, histograms for the Teifi and Usk were bimodal with frequency maxima at 61, 63 and 62, 64 respectively (Fig. 2). Although previous workers have reported no significant differences in myotome numbers for the adults of *L. planeri* and *L. fluviatilis*[7–10], it was thought possible that the bimodal distributions might be due to small differences in the myotome numbers of the ammocoetes. An examination was therefore made of the gonads in two groups of ammocoetes from the Teifi and Usk, falling within the upper and lower ranges of myotome numbers. Of eleven female ammocoetes with 64–66 trunk myotomes three were of the *fluviatilis* type with more than 65 oocytes/section; the other group of twelve animals with 59–63 myotomes contained one ammocoete of this species with 110 oocytes/section. Since *planeri* ammocoetes were found over the whole range of myotome numbers (59–66) there is no evidence for differences in the distribution of myotome numbers in the two species.

Although the method of identification based on oocyte numbers has obvious limitations, it is quite possible that in older ammocoetes the males also could be differentiated by the number of spermatogonial follicles and the relative size of the testis. In any event the ability to identify at least the female ammocoetes provides the necessary basis for further attempts to discover other more convenient diagnostic characters.

M. W. Hardisty

College of Science and Technology, Bristol.

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Warm-water Species in the Plankton off Newfoundland during Winter Months

Since September 1959 plankton samples have been taken, at monthly intervals whenever possible, with the Continuous Plankton Recorder[1] between Iceland and Newfoundland. The Recorder is towed by commercial ships and obtains a continuous sample of plankton from a depth of 10 m. This sampling is part of a regular survey of the plankton of the North Atlantic Ocean and North Sea conducted from this Laboratory.

The western portions of the Newfoundland Recorder routes during the winter months of 1959–60 are shown in Fig. 1. In this sea area, boreal species, particularly the copepod *Calanus finmarchicus* and the euphausiid *Thysanoessa longicaudata*, usually constitute almost the whole of the zooplankton sampled by the Recorder. During December 1959, however, many *Calanus* present in the region off the Belle Isle Strait agreed with the description by Jaschnov[2] of the stage V copepodites of *Calanus glacialis*, an arctic form. In February 1960, the sampling followed a course towards Cape Race, and a clearly defined patch of warm-water species was found off the north-eastern slope of the Grand Banks. These included the copepods *Nannocalanus minor*, *Clausocalanus arcuicornis*, *Lucicutia flavicornis*, *Pleuromamma gracilis*, *Pleuromamma borealis* and *Heterorhabdus spinifrons*, together with the euphausiids *Thysanoessa gregaria* and *Euphausia krohnii*. The record for March followed closely a similar course to that taken during February; no warm-water species were observed but two species normally associated with cold-water conditions, the copepod *Metridia longa* and the chætognath *Sagitta maxima*, appeared in the same area. In April the Recorder was towed along a more southerly course and again no more warm-water species were encountered, but two patches of cold-water copepods, *Metridia longa* and *Calanus hyperboreus*, were found.

During early March 1961, a patch of warm-water species was again found, on this occasion between 52° 00′ N., 42° 25′ W. and 50° 20′ N., 43° 25′ W., that is, between 90 and 140 miles east of the patch observed during February 1960.

The occurrence of warm-water species in February and March, when surface temperatures in the region are usually close to the annual minimum[3], is related presumably to the changing pattern of water movement.

The area east of Newfoundland is influenced, from the north, by the cold water of the Labrador Current and, from the south, by the warmer waters of the North Atlantic Current, once thought to be formed by a branching of the Gulf Stream System[4]. Fuglister[5,6] has suggested that there is no real branching; but that the Gulf Stream System in this region consists of a number of disconnected filaments of current the pattern of which is variable.

The presence of warm-water species to the north-east of the Grand Banks, therefore, may indicate a temporary extension of the Gulf Stream System. It is not known whether such events occur at regular or irregular intervals, but it is interesting to note that Kielhorn[7] found several warm-water species of zooplankton in January and February at Weather Station *B* in the Labrador Sea during a survey throughout 1950.

Stommel[8] has directed attention to the lack of knowledge about fluctuations in ocean currents,

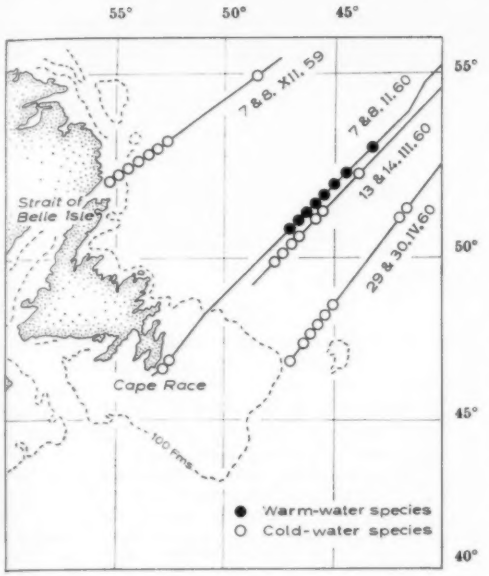

Fig. 1. Recorder routes in the region of the Newfoundland Grand Banks, during December 1959–April 1960

an omission which can only be remedied by long-term oceanographic surveys. Regular sampling with the Continuous Plankton Recorder between Iceland and Newfoundland may help to provide information on the variations and irregularities in the current system of the western North Atlantic.

I am most grateful to the staff of the Icelandic Fisheries Laboratory and to the ships' crews of the Icelandic Steamship Co. for their help in obtaining the samples.

V. Bainbridge

Oceanographic Laboratory,
Edinburgh.

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Effect of Ferric Dimethyldithiocarbamate on the Rooting of Cuttings of *Hevea brasiliensis*

A method involving the technique of mist-propagation which has led to the successful rooting of leafy clonal cuttings of the Para rubber tree, *Hevea brasiliensis*, has been reported earlier[1,2]. A routine application of 'Fermate' dust (ferric dimethyldithiocarbamate, kindly supplied by Messrs. E. I. Dupont de Nemours and Co., Inc.) to the base of the cuttings was mentioned in these publications and elsewhere by Levandowsky[3] also working with *Hevea* cuttings.

This compound was used as a fungicide to protect the trimmed base of the cutting which might be subject to fungal attack; but later observations showed that rotting of the stem was not usually severe, even on untreated cuttings under mist-propagation conditions, and poor rooting was often due to the deterioration of the leaves, which resulted from a long period under the spray. Improved rooting where this compound was used could not therefore be attributed entirely to its fungicidal effect and recent work has established its importance in stimulating initiation of roots in *Hevea* cuttings. Some of the evidence is presented here.

After trimming, the cuttings were stood in water for a few minutes to coagulate the latex, which is then peeled off; the bases were then dipped into the dithiocarbamate powder (diluted in talc to give 35 per cent of active ingredient) to a depth of 1 in. and immediately planted into the rooting beds containing a 1 : 1 mixture of sawdust and sand. Talc was used as the control and treatments replicated at least three times. After forty days under mist, periodical inspections for rooting were made; dead cuttings or those in poor condition due to deterioration of the leaves were discarded. Table 1 shows the rooting records from some of these experiments.

Table 1 shows that clones *RRIM* 501, 605 and 632, *AVROS* 157 and *PB* 5/63 show little or no success where untreated, but application of 'Fermate' improves rooting significantly at all stages. With *GT* 1 (two experiments) and *PB* 86 there is good final success with both treatments, but rooting is markedly accelerated where 'Fermate' is applied.

Three concentrations of 'Fermate' and the talc control were compared in two experiments with clones *PB* 86 and *RRIM* 501; the rooting records are shown graphically in Figs. 1 and 2.

Fig. 1 shows that all three concentrations of 'Fermate' accelerated the rate of rooting of *PB* 86 cuttings relative to the talc control. This is most marked with the 75 per cent powder treatment, which achieves 100 per cent success after 75 days. After 98 days, this treatment is significantly better than the control, and the 17·5 per cent concentration.

Fig. 2 illustrates the rooting of *RRIM* 501 cuttings treated similarly. Again, the 75 per cent concentration results in greatest final success and induces earlier rooting. After 114 days, all three levels of 'Fermate' show significantly greater success than the control, which has given only 10 per cent final success.

'Manzate', 'Zerlate' and 'Parzate', manganese ethylenebisdithiocarbamate, zinc dimethyldithiocarbamate and zinc ethylenebisdithiocarbamate respectively, all at 40 per cent concentration, were used in comparison with 40 per cent 'Fermate' and talc in one experiment. All four compounds led to significant increases in rooting success relative to the talc control. In final success the superiority of 'Fermate' over 'Manzate' and 'Parzate' was not established, although it was significantly better than 'Zerlate'.

Preliminary experiments using growth substances including indole-3-butyric acid and naphthalene acetic acid have proved unsuccessful in promoting root initiation in leafy clonal *Hevea* cuttings, but more experiments with these compounds and with other dithiocarbamates are in progress and will be reported in detail elsewhere.

Van der Kerk *et al.*[5] first reported that the compound *S*-carboxy-methyl dimethyldithiocarbamate showed growth-regulating activity. Ng and Carr[4] also showed that this compound at an optimal concen-

Table 1. ROOTING OF SEVEN *Hevea brasiliensis* CLONES WITH AND WITHOUT 'FERMATE' TREATMENT

Clone	Treatment	No. of cuttings	No. rooted. Cumulative totals						
			Days after insertion						
			40	50	60	70	80	98	114
RRIM 501	Talc	40	—	0	0	2	3	3	4 (10%)
	Fermate	40	—	3	3	12	14	15	18 (45%)
RRIM 605	Talc	21	0	0	0	—	—	—	—
	Fermate	21	10	13	14 (67%)	—	—	—	
RRIM 632	Talc	21	0	0	0	1	1 (5%)	—	
	Fermate	21	10	13	14	15	17 (81%)	—	
AVROS 157	Talc	21	0	0	0	1	2 (10%)	—	
	Fermate	21	6	13	16	16	19 (90%)	—	
PB 5/63	Talc	21	0	0	0	—	—	—	
	Fermate	21	5	7	7	9	10 (48%)	—	
GT 1	Talc	21	1	9	18	20 (95%)	—	—	
	Fermate	21	11	21 (100%)	—	—	—	—	
GT 1	Talc	27	2	8	17	20	21 (78%)	—	
	Fermate	27	8	17	24	24	25 (93%)	—	
PB 86	Talc	36	1	8	15	22	26	29 (82%)	
	Fermate	36	12	24	28	30	30	31 (88%)	

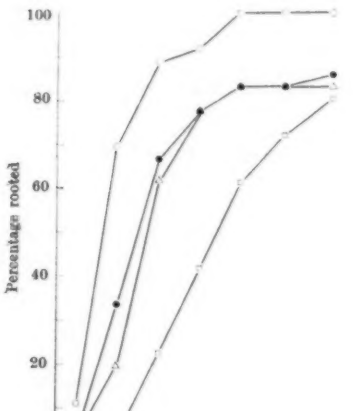

Fig. 1. Effect of three concentrations of 'Fermate' on the rooting of *PB* 86 cuttings (3 × 12 cuttings per treatment)

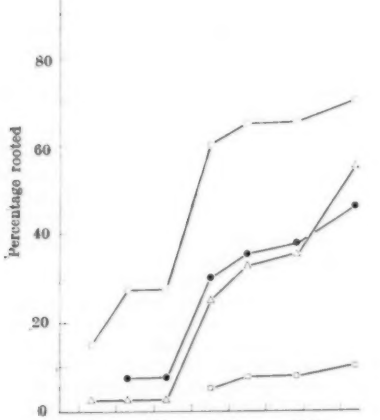

Fig. 2. Effect of three concentrations of 'Fermate' on the rooting of *BRIM* 501 cuttings (4 × 10 cuttings per treatment). Talc control: 17·5, 35 and 75 per cent 'Fermate'

tration of 20 mgm./l. effected a stimulation of growth of wheat coleoptile segments and had an activity of the same order as indole-3-acetic acid.

In our experiments, related dithiocarbamate compounds have been used at extremely high concentrations and have shown a marked effect on root initiation which cannot be completely explained by their fungicidal action.

G. H. TINLEY

Rubber Research Institute of Malaya,
Kuala Lumpur,
Federation of Malaya.

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Possibilities of Vegetative Propagation in the Coconut (*Cocos nucifera* L.)

THE coconut is a perennial palm which takes 6–8 years to begin bearing, but later continues to bear right through the year and all through its life, extending during a period of 60 years or more depending on local conditions. It is highly cross-pollinated in Nature, particularly the tall variety, and is propagated only through seed. These features, namely, the long interval between generations and the highly heterogeneous nature of the plant population, are proving to be great handicaps in making quick headway in coconut breeding. The perennial nature of the palm, on the other hand, is of advantage, in that the benefit from any improvement can be enjoyed over a very long period.

Vegetative propagation is the one sure method of propagating lines true to type, and it could be very valuable in coconut research, as has been recently pointed out (refs. 1–3). In the coconut, which is a monocotyledonous plant devoid of cambium, the prospect of vegetative propagation is at present remote, but there are indications that the problem is worth following up. In Nature, tendencies on the part of the palm to produce aerial roots under adverse conditions in the environment and/or diseased conditions of the palms have been noticed[4]. Rare cases of the palms producing suckers have also been reported[5,6]. If these could be produced at will, some definite progress in vegetative propagation of coconut

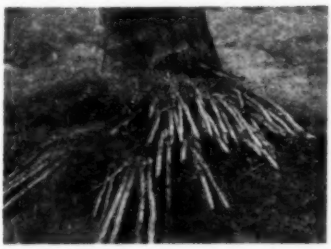

Fig. 1. Roots formed at the girdled portion on the coconut stem above ground-level where the growth substances were applied

would have been made. That rooting can be artificially induced in the stem of the coconut palm above ground-level through marcottage, has been reported[4]. After the formation of large numbers of roots, the crown can be separated by severing the palm just below the rooted region and planting elsewhere. This procedure, of course, does not permit the multiplication of desirable palms, but may help to rejuvenate and to maintain them for a much longer period than otherwise.

It is now well established that use of appropriate growth regulators in optimum concentrations can induce rooting and other meristematic activity in a variety of plants. In order to investigate how far their use in coconut will be helpful in inducing meristematic activity leading to rooting and probably suckering too, trials were carried out at this Research Station with mixtures of selected growth regulators, namely, α-naphthalene acetic acid, indole-3-butyric acid and certain purine compounds such as adenine, adenosine, alloxanthine and uric acid, in different concentrations and proportions. The growth regulators were applied as an aqueous spray or in lanolin paste after girdling or debarking the stem near ground-level. The points of application were then covered with soil which was kept moist during the rainless period by artificial watering. Initiation of roots was observed in about 1 yr. in the case of the following formulations only and roots were profuse (Fig. 1) in about eighteen months: 1 per cent indole-3-butyric acid in lanolin; 0·05 per cent naphthalene acetic acid in lanolin; and a mixture of 20 ml. of 80 p.p.m. adenosine, 5 ml. of 20 p.p.m. naphthalene acetic acid and 15 ml. of water applied as spray. There was no sign of suckering seen in any of the treatments so far.

These results thus indicate that inducing meristematic activity in the coconut is possible. Further systematic trials with a larger number of important growth regulators alone and in combinations and mixed in different proportions are under way to assess their relative merits.

Our thanks are due to Mr. A. K. Kamalakaran for his assistance.

S. R. Gangolly
K. M. Pandalai

Central Coconut Research Station,
Kasaragod, S. India.

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Effect of Red and Far-red Irradiation on the Germination Process in Seeds of *Pinus sylvestris* L.

Since the first work on the red – far-red reversible mechanism controlling the germination of lettuce seeds[1], many investigations have shown the same type of light-controlled germination in seeds of herbaceous plants as well as in tree seeds[2]. In the genus *Pinus* it has been reported for *P. virginiana* Mill.[3], *P. taeda* L.[4] and earlier by me for *P. sylvestris* L.[5]. Results from further work on the red – far-red controlled germination in seeds of *Pinus sylvestris* L. are described here.

The germination experiments were carried out in Jacobsen apparatuses at + 25° C. during 20 days. For the irradiations the following equipment was used: red irradiation from red fluorescent tubes (Philips *TL*/15, emission range between 5800 and 7200 Å. with a sharp maximum at 6600 Å.), far-red irradiation from a 500-W. incandescent lamp with a 10-cm. water filter and double layers of blue and red 'Cellophane' (transmission from 6800 Å. and upwards together with some transmission in blue). The intensity of both sources was $3{\cdot}5 \times 10^3$ ergs/cm.^{2} sec. The time of irradiation, the same for all the experiments here reported, was 30 min. The irradiations were started after 6 hr. imbibition. The respiration measurements were made by the Warburg direct method. Cell divisions in the seeds were examined by aceto-orcein staining of embryo smears[6]. All handling of the seed materials was performed in green safe-light[7].

As in lettuce seeds, the light-requirement for germination can be satisfied by a single irradiation. Maximum sensitivity is obtained after 6–12 hr. imbibition. As reported earlier[5,6], seeds which have not imbibed water can also be influenced by irradiation. From the action spectrum it is evident that red light around 6600 Å. is most effective in stimulating germination. Stimulation can be nullified by far-red irradiation immediately afterwards (see Fig. 1). With a time-lapse of 48 hr. between a red and a far-red irradiation, the latter became completely ineffective. At that time, all seed embryos investigated showed mitoses. The seed temperature between the red and far-red light impulses was of importance, but not the temperature during the irradiations. The light-requirement of the seeds for germination depends on seed coat being intact. After a small puncture or complete removal of the seed coats, germination in the dark became high. It was then impossible to inhibit germination by far-red irradiation. The same results were obtained after stratification of the intact seeds (storage on wet cotton pads in Petri dishes at + 2–4° C. in the dark for 2 weeks).

In order to establish whether the red – far-red irradiations influence any phases of the germination process earlier than the visible germination, work on seed respiration and on the occurrence of mitoses in seed embryos was carried out during the first three days of the germination period. Some of the results are presented in Fig. 1.

The respiration measurements shown in Fig. 1 were started after 48 hr. imbibition, that is, 42 hr. after the start of irradiation, and similarly for the work on mitoses. The values for respiration are based on 3 × 50 seeds per experiment and expressed in μlitres oxygen/50 seeds and ½ hr. The values for mitoses are the percentages of embryos investigated with mitoses determined on 2 × 10 embryos per

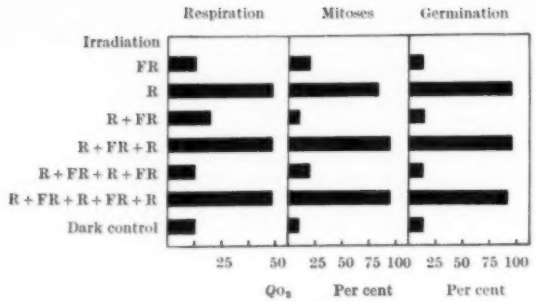

Fig. 1. Reversible effects of red (R) and far-red (FR) irradiations on the respiration-rate, the occurrence of mitoses in embryos and the percentage of germination in seeds of *Pinus sylvestris* L.

experiment. The germination values are based on 2 × (100–10) seeds per experiment (after 20 days). The stimulating effect of red irradiation is significant ($0{\cdot}001 > P$) in all the three groups of experiments. From these results it is evident that the effect of red and far-red irradiations on the germination can be traced quite early in the germination process. Red light stimulates, and far-red light inhibits, respiration as well as mitotic activity in the embryos. A significant increase in respiratory activity induced by red light can be observed after imbibition for 24 hr. and stimulation of mitotic activity after 36 hr. The visible signs of germination (protruding rootlets) appear first after more than 48 hr. imbibition. In seed samples irradiated with red + far-red the inhibited visible germination can be traced in an inhibition of respiration and mitotic activity. In recently published work on lettuce seeds[9] it has been stressed that the occurrence of mitoses in the embryos is independent of the red – far-red irradiation, as is also the respiration of the seeds before the appearance of the first visible signs of germination. This is evidently not the case for the seeds investigated here, which in many other respects show the same responses as lettuce seeds.

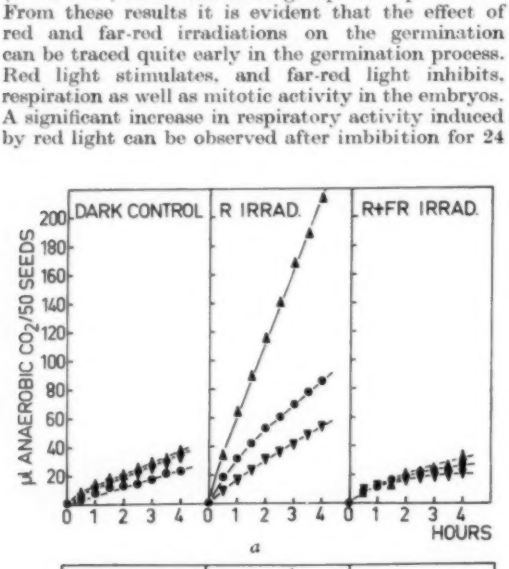

Fig. 2. Reversible effect of red and far-red irradiations on emission of carbon dioxide from seeds of *Pinus sylvestris* L. under anaerobic (*a*) and aerobic (*b*) conditions. The measurements were made after 24 hr. (inverted triangles), 48 hr. (dots) and 72 hr. (triangles) imbibition

In other respiration measurements the effects of red and far-red irradiations on the emission of carbon dioxide under aerobic and anaerobic conditions have been investigated after different times of imbibition. The results are given in Fig. 2.

For both aerobic and anaerobic conditions, the rate of emission of carbon dioxide is already higher after 24 hr. in the red-irradiated seeds as compared with the dark control and the red + far-red irradiated seeds ($0{\cdot}001 > P$). There is thus an effect of irradiation, not only on the aerobic but also on the anaerobic phase of the seed respiration. The effect on the anaerobic phase can perhaps be combined with the red – far-red effect in the synthesis of the TPN-linked triosephosphate dehydrogenase[10].

The results will be published in detail elsewhere. This work has been supported by a grant from the Swedish Forest Research Foundation.

Bengt Nyman

Institute of Forest Botany,
Royal School of Forestry,
Stockholm.

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MICROBIOLOGY

Numbers and Types of Bacteria from the Rhizosphere of Pasture Plants : Possible Occurrence of *L*-forms

During work on micro-organisms in meadow and pasture soils[1] large numbers and high activity were found in the surface soil layer of 0–5 cm. I decided to investigate the plant roots which form a dense mat in this zone since these might influence micro-organisms. Microscopical counts and estimations of the quantity of fungal and actinomycete mycelia in the soil between the roots and in soil washed from the roots were carried out using stained agar films as Jones and Mollison did, but with phase-contrast observation[2,3]. Agar plate counts were used as a second method, yielding various results, and an attempt to explain this is the purpose of this communication.

Table 1. Numbers of Bacteria in the Rhizosphere of *Holcus lanatus* and Surface Soil

Date	Log dilution	Sandy loam, dry *Holcus lanatus* soil No. × 10^9		Clay, moist *Holcus lanatus* soil No. × 10^9	
12.11.1957	7	170	1·50	156	1·25
	8	—	—	616	
20.1.1958	7	565	0·25	190	0·37
	8	4,430		1,260	
23.4.1958	7	367	1·24	155	0·99
	8	2,810		866	
3.6.1958	7	1,980	1·09	420	0·68
	8	2,610		2,490	
16.9.1958	7	563	1·22	1,080	1·36
	8	1,490		8,360	

Samples were collected from two locations. The suspensions from 0–5 cm. surface soil and roots obtained by vigorous shaking for 5 min. with sterilized tap-water were diluted by successive steps of 1:10 to 1:10^7 or 1:10^8 and plated with soil extract agar cooled to 45° C. When collected the day before, the samples were kept at +2–4° C. in a refrigerator to avoid multiplication of the bacteria.

Table 1 shows the numbers of bacteria (average values from five replicate plates) in the rhizosphere of *Holcus lanatus* on five dates as an example for twelve species of plants investigated. These were: *Dactylis glomerata*, *Helictotrichon pubescens*, *Poa pratensis*, *Trifolium repens*, *Plantago lanceolata*, *Achillea millefolium*, *Galium mollugo*, *Rumex acetosa*, in a meadow on sandy loam, and *Alopecurus pratensis*. *Festuca pratensis*, and *Ranunculus acris* in a moister meadow on clay soil. *Holcus lanatus* was collected from both locations. The magnitude of bacterial numbers is surprising and has not been reported before. It reached 8 × 10^{12}/gm. and depended largely on the logarithm of the dilution. In contrast the values for the soils are much lower, between 1 and 2 × 10^9/gm. The maximum number of rhizospheres in sandy loam soil occurred in winter when the soil population was a minimum whereas in the clay soil rhizosphere maximum was observed in early autumn when soil bacteria also reached their greatest number.

These results exclude the assumption of normally developed bacteria occurring in such large numbers. Assuming a minimum size for a small coccoid soil form of 0·125μ^3, 8 × 10^{12} of these would fill completely 1 cm.^{3} without leaving any space between them. Even if the sample consisted entirely of bacteria of this size it could contain only about 4 × 10^{12}/gm. Experimental error, on the other hand, could be excluded because the control plates showed no colony growth in any case. Plates inoculated with diluted rhizosphere suspension showed even with logarithm of dilution 8 up to 2,000 colonies per plate, which could be counted with reasonable precision using a binocular microscope. Agar films gave maximal numbers between 300 and 400 × 10^9/gm. in the rhizosphere of *Holcus lanatus*, which had the highest numbers among all plants investigated. This is in any event considerably lower than the plate counts, which normally yield lower numbers than direct counts.

These high numbers could only be caused by very small propagation units known as '*L*-forms' of 0·1–0·2μ diameter. Küster[4] obtained them from soil bacteria after treatment with penicillin or glycine and Vilas[5] identified such *L*-forms in soil. The occurrence of filterable *L*-forms in the rhizosphere of the plants investigated may be postulated. It may be favoured by effects originating from the dense population of microbes occurring in a naturally grown grass sward.

The main group of bacteria obtained belonged to *Arthrobacter*, a genus of Conn and Dimmick, 1931, autochthonous coryneform soil bacteria. They are Gram-positive to Gram-variable rods of different length, which form either smaller ovoid cells of 0·5–0·8μ diameter or larger cells in terminal swellings of 1·0–1·2μ diameter, the so-called cystites, within their cell walls. These may grow and form rods again[6,7]. Their proportion in soil and rhizosphere decreases more or less during the summer in favour of Gram-positive and Gram-negative rods. The latter are pseudomonas-like, motile short rods, some of them fluorescent and long, thin rods of 10–20μ length, which usually show a slowly creeping movement. These long rods reached the high proportion of 26 per cent only in the rhizosphere of *Trifolium repens*. In accordance with the results of Lochhead[8], the rhizosphere generally contained relatively more motile and Gram-negative bacteria than the soil. This selective rhizosphere effect was most marked with *Trifolium repens* and least with *Alopecurus pratensis*. Probably the most numerous *Arthrobacter* forms and the Gram-negative rods would provide the *L*-forms causing the high counts.

Table 2. Percentage Composition of Bacterial Population Isolated from Soil and Rhizosphere Dilution Plates

Date	Source	No. of isolates	*Arthrobacter*	*Mycobacterium*	Gram-positive rods	Gram-negative rods	Cocci	Motile bacteria
12.11.1957	*Trifolium repens*	100	74	17	—	1	8	
12.11.1957	*Ranunculus acris*	40	91	6	2	1	—	
12.11.1957	*Alopecurus pratensis*	50	88	—	4	2	6	
3.6.1958	Sandy loam, dry	51	48	4	16	32	—	18
3.6.1958	*Trifolium repens*	59	35	3	5	57	—	33
3.6.1958	Clay, moist	53	57	2	8	24	9	11
3.6.1958	*Ranunculus acris*	54	48	5	15	32	—	37
3.6.1958	*Alopecurus pratensis*	52	75	—	12	13	—	19

G. Jagnow*

Institut für Grünlandwirtschaft und Futterbau,
J. Liebig-University,
Giessen.

* Present address: Gezira Research Farm, Wad Medani, Sudan.

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Morphological Variation in *Ferrobacillus ferrooxidans* related to the Rate of Iron Oxidation

While investigating the removal of pyritic sulphur from coal by a microbiological method, we propagated large numbers of the chemoautotrophic iron-oxidizing bacterium *Ferrobacillus ferrooxidans*. During mass culture of the bacterium in an inorganic iron salts

medium (9K)[1], it was observed that alterations in cellular morphology occurred in response to excessively vigorous aeration. Normal cells measure 1·0–1·7μ by 0·5μ (Fig. 1A), while the abnormal (morphologically altered) cells differed by having a diameter of 1·0μ, so that many enlarged, coccoidal forms were present (Fig. 1B). The abnormal strain, on large-scale sub-culture using less vigorous aeration, or in shake-culture flasks, showed virtually complete reversion to the normal morphology after the second transfer (Fig. 1C, D).

Normal (Fig. 1A), abnormal (Fig. 1B) and reverted (Fig. 1D) cells were collected as previously described[1] and the rates at which they oxidized ferrous to ferric iron were determined by standard manometric techniques. Each Warburg vessel contained a total of 1·7 ml. liquid consisting of 500 μM Fe^{++} (as $FeSO_4.7H_2O$ dissolved in distilled water acidified to *p*H 2·6 with sulphuric acid) in the main compartment, 0·5 ml. of cell suspension in the side arm, and 0·2 ml. of 20 per cent potassium hydroxide in the centre well. The gas phase was air. All suspensions were tipped in at zero time and the uptake of oxygen at 30° C. was measured at 10-min. intervals over a period of 70–120 min. Cellular nitrogen was determined by a micro-Kjeldahl procedure.

The results indicated that abnormal cells were consistently less active than their normal or reverted counterparts. However, $Qo_2(N)$ values (μl. oxygen uptake/mgm. cellular nitrogen/hr.) varied considerably with cell concentration within each of the resting cell suspensions. A more systematic investigation was undertaken where the rate of iron oxidation by normal, abnormal and reverted cells was examined as a function of cellular nitrogen. The results are shown in Fig. 2. The rate of oxidation of the three

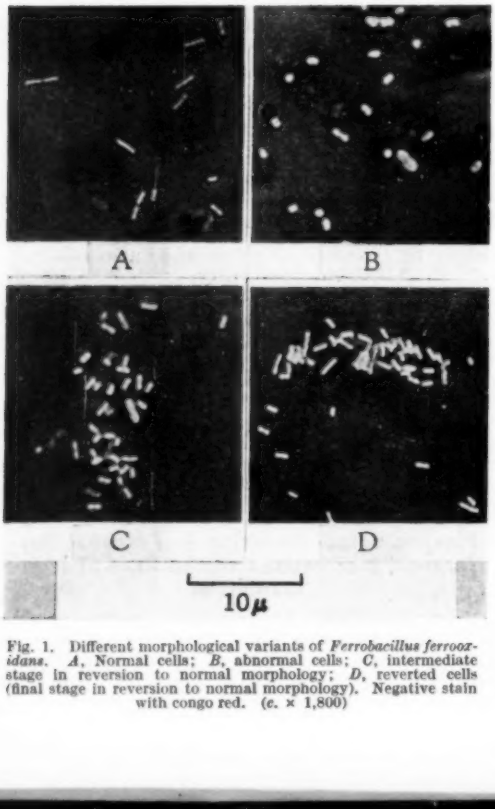

Fig. 1. Different morphological variants of *Ferrobacillus ferrooxidans*. *A*, Normal cells; *B*, abnormal cells; *C*, intermediate stage in reversion to normal morphology; *D*, reverted cells (final stage in reversion to normal morphology). Negative stain with congo red. (*c.* × 1,800)

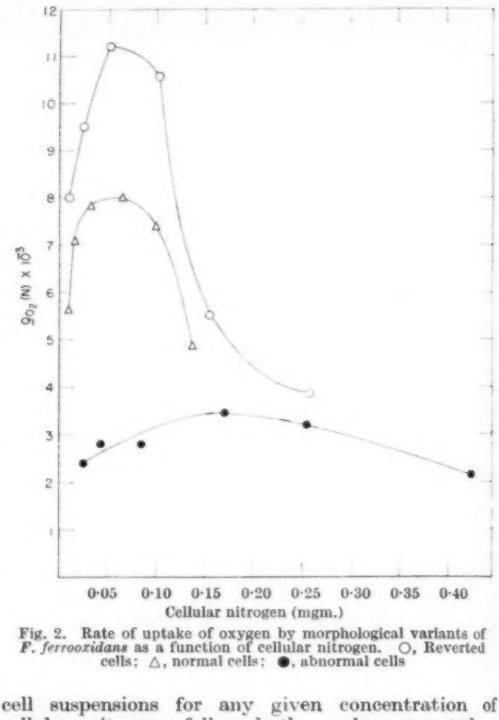

Fig. 2. Rate of uptake of oxygen by morphological variants of *F. ferrooxidans* as a function of cellular nitrogen. ○, Reverted cells; △, normal cells; ●, abnormal cells

cell suspensions for any given concentration of cellular nitrogen followed the order—reverted > normal > abnormal. Maximum and minimum $Qo_2(N)$ values ranged from 11,209 (0·051 mgm. nitrogen) to 3,840 (0·257 mgm. nitrogen) for reverted cells, 8,012 (0·065 mgm. nitrogen) to 4,784 (0·162 mgm. nitrogen) for normal cells, and 3,480 (0·170 mgm. nitrogen) to 1,932 (0·425 mgm. nitrogen) for abnormal cells.

Beck[2] reported that an iron-oxidizing bacterium, isolated from the acid water of Bingham Canyon, Utah, exhibited $Qo_2(N)$ values that decreased with increasing concentrations of cells. He attributed this to the possible failure of oxygen equilibrium between the fluid and gaseous phases during rapid oxygen uptake. Our findings might be explained on this assumption up to the maximum $Qo_2(N)$ values, but the decrease in $Qo_2(N)$, on further dilution of the cells, argues against oxygen solubility as the sole factor in determining the curves illustrated in Fig. 2.

On the assumption that the nitrogenous constituents of the larger, more spherical, abnormal cells increased in proportion to the increased cell volume, it is interesting to speculate as to the site(s) of iron oxidation in this bacterium. The smaller surface-to-volume ratio of abnormal cells, coupled with the lower rate of oxidation, seems to imply that the cell surface may play an active part in the oxidation of iron, and that at least one, and possibly more, of the unknown steps in the transfer of electrons from ferrous iron to oxygen may occur at this site.

M. P. Silverman
M. H. Rogoff

Bureau of Mines,
4800 Forbes Avenue,
Pittsburgh 13, Penn.

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Simple, Quick Methods for Detection of 6-Amino-penicillanic Acid

ONE of the greatest discoveries of the past years in penicillin research was the discovery, isolation[1] and preparation of 6-amino-penicillanic acid (6-APA) forming the common 'nucleus' of natural penicillins[2,3], and the successful introduction into therapy of semi-synthetic penicillins produced from it[4,5]. The new semi-synthetic penicillin preparations have already made great progress in the fight against resistant bacteria. The possibility is now laid open for the preparation of penicillins possessing novel effectiveness[6]. The basis for efforts of this kind is 6-APA research, and its quick identification in fermentation liquor and other reaction mixtures.

The biological methods hitherto used (such as paper-chromatography; the chemical transformation of 6-APA into some familiar natural penicillin, or bioassay) even if not complicated, are comparatively lengthy. The following two simple methods have been devised for quick detection of 6-APA:

(1) The essence of the paper-slice method is the cutting of 1 cm.-wide slices from Whatman No. 1 filter-paper in appropriate lengths and washing them first in a solution of 3 per cent sodium bicarbonate, then, after drying, in a solution of 1 per cent phenylacetylchloride in acetone. After drying in air, a solution containing 6-APA is added, drop-wise, to the impregnated paper. If there is 6-APA in the solution, it will be converted to *G*-penicillin on the paper and yield an inhibition zone. This method is appropriate for qualitative determination.

(2) The 6-APA content of fermentation liquid or other solutions is demonstrable also by means of the hole plate method. 6-APA is transformed directly into a natural penicillin within holes in an agar plate inoculated with *B. subtilis* spores. In this manner, the time and effort needed to perform this reaction in a separate test-tube are saved. Fig. 1 illustrates such a demonstration. Each fermentation liquid is measured into two holes. Into one hole (upper row in the figure) a single drop of 3 per cent $NaHCO_3$ solution is added, while into the other hole (lower row in the figure) one drop of 1 per cent phenylacetylchloride in acetone is also added. The plate is then incubated for 5 hr. at 37° C. If there is 6-APA in the fermentation liquid, it is converted to *G*-penicillin and yields an inhibition zone, as can be seen around the holes 1–4 of the lower row in the figure. Around the holes of the upper row is the inhibition zone due to the penicillin remaining in the fermentation fluid after extraction. The fifth hole, where distilled water was introduced, the reagents in the quantity applied do not produce an inhibition zone. This method is applicable for the quantitative, quick detection even of a tenth unit of 6-APA.

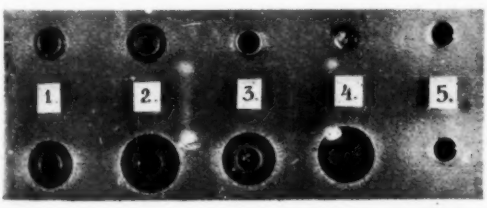

Fig. 1. Detection of 6-APA content of fermentation liquids by use of the hole plate method.

Test micro-organism: *B. subtilis*, 0·1 ml. of the liquid containing 6-APA (1, 0·04 unit; 2, 0·2 unit; 3, 0·06 unit; 4, 0·2 unit; 5, dist.H_2O) measured into holes. One drop of 3 per cent $NaHCO_3$, and, on the lower row, an additional drop of 1 per cent phenylacetylchloride in acetone is measured. In the upper row: inhibition zone of remaining natural penicillins. In the lower one: inhibition zone of *G*-penicillin deriving from 6-APA

J. URI*

Department of Pharmacology,
University Medical School,

F. SZTARICSKAI

'Biogal' Pharmaceutic Factory,
Debrecen, Hungary.

* Present address: Department of Biochemistry, London School of Hygiene and Tropical Medicine, Keppel Street, London, W.C.1.

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ENTOMOLOGY

Exchanges of Sodium Ions in the Central Nervous System of an Insect (*Periplaneta americana* L.)

IN earlier work it was shown that the exchanges of sodium and potassium ions between the hæmolymph and the central nervous system of the cockroach occurred relatively rapidly[1]. It was concluded from these results that the connective tissue layer surrounding the nervous system did not function as a significant diffusion barrier and that in such a dynamic system any selective permeability must be attributed to the underlying cellular layer of the nerve sheath or some associated elements.

An attempt has recently been made to throw some further light on these processes by studying the efflux of sodium ions from isolated abdominal nerve cords and single connectives and ganglia of *Periplaneta americana* L. In these experiments the rate of loss of sodium-24 was measured by determining the decline in radioactivity of isolated preparations maintained in a flowing physiological solution. The greater part of the efflux of sodium-24 measured in this way was found to approximate to a simple exponential function, both for the whole nerve cord and for the isolated fragments. The presence of 2:4-dinitrophenol and cyanide in low concentrations was found to reduce substantially the rate of efflux of sodium measured in these experiments (Fig. 1). Similarly, an absence of potassium ions from the external solution resulted in a slowing down of loss of sodium, although sodium-free solutions appeared to have no appreciable effects on the measured efflux from the nerve cord. Thus it seems that, as for some other cells and tissues[2], sodium ions are extruded from the central nervous system by a metabolically maintained secretory mechanism which is also associated with the uptake of potassium ions.

In another group of experiments the effect of removing the cellular and fibrous nerve sheath on the rate of sodium extrusion was investigated. It was found that the rate of efflux of sodium-24 from the terminal abdominal ganglion was not appreciably affected by the removal of substantial portions of the perilemma (Fig. 2). It must be concluded, then, that the rate-limiting process measured in these experiments was not the transfer of the ions across

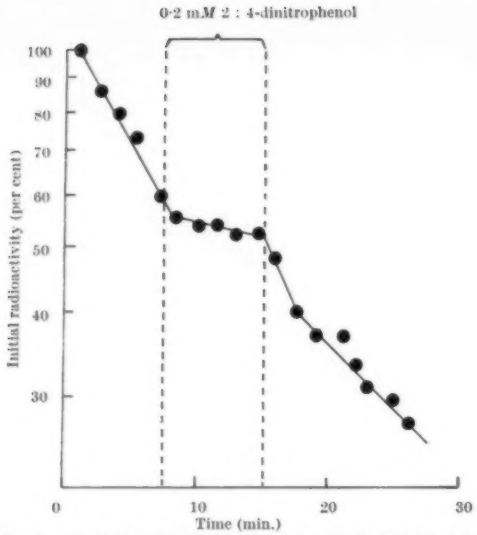

Fig. 1. Effect of 0·2 m*M* 2 : 4-dinitrophenol on the rate of loss of sodium-24 from a single, ligatured, terminal abdominal ganglion washed in inactive physiological solution

the cellular perineurium, but the exchanges associated with some underlying cellular components of the central nervous system. The relatively rapid effects of the poison molecules and the potassium-free solutions on the measured effluxes from the intact abdominal nerve cord thus imply that the changes in the chemical composition of the external solution were quickly transmitted to the deeper layers of the central nervous system.

The ionic fluxes calculated in the previous account[1], using conventional equations, involved the assumption that the rate-limiting process was the transfer across the superficial boundary of the central nervous system and that the movements within the underlying tissues occurred relatively rapidly so that the labelled ions were effectively well mixed. The results here show that this represents an over-simplification, and consequently the calculated values have little significance. It is hoped that in a future investigation, in which any relatively rapid movements can be measured, a quantitative analysis can be given for this more complex system.

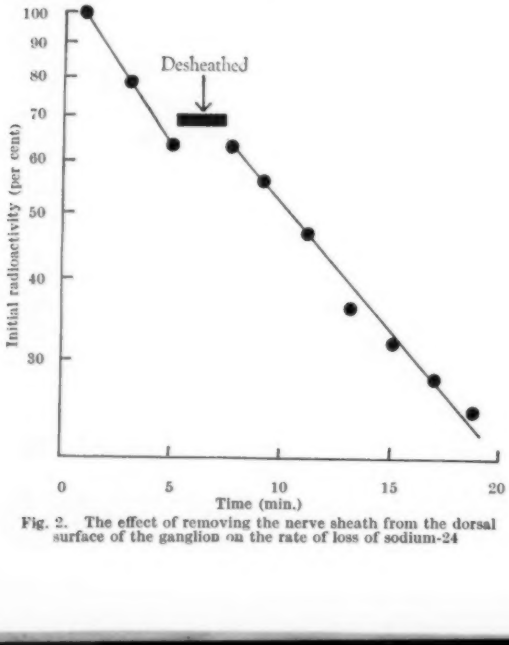

Fig. 2. The effect of removing the nerve sheath from the dorsal surface of the ganglion on the rate of loss of sodium-24

J. E. Treherne

Agricultural Research Council
Unit of Insect Physiology,
Department of Zoology,
University of Cambridge.

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An Acid Mucopolysaccharide in Cockroach Ganglia

In a recent histochemical investigation of the neurones and connective tissues of the meso- and meta-thoracic ganglia of the cockroach, *Periplaneta americana*[1], it was noticed that an area surrounding the central mass of nerve fibres stained metachromatically with toluidine blue. The region separates the neurones and connective tissue sheath from the main mass of nerve fibres. There is no comparable region in the interganglionic connectives or peripheral nerves. This seems at present to be a structure peculiar to *Periplaneta* and other cockroaches; a similar region has been seen after staining with toluidine blue in *Blaberus craniifer*, *Blatta orientalis*, *Blattella germanica* and *Diploptera punctata*. No such region is found in insects of other orders which have been studied histochemically, for example, *Ephestia kühniella* (Richards, A. G., personal communication), *Locusta migratoria* (unpublished observations) and sucking lice[2].

The metachromatic substance in *Periplaneta* has no apparent structure and appears to be extra-cellular; the inner and outer borders are delimited by the glial cells which send slender processes across it, and, in addition, occasional glial cell bodies are seen. This is the appearance in sections of ganglia fixed in Zenker's fluid (Fig. 1). If, however, a section from a ganglion fixed in formalin is stained with toluidine blue, the metachromatic substance is displaced inwards; the region is seen to be disrupted and the substance now lies on the surface of the broken glial processes around the central nerve fibre mass. This appearance is described by Hess[3], who stained sections of these ganglia with cresyl violet, after fixation in either Bodian's or Bouin's fluids. He describes the metachromatic cytoplasm as forming a 'reticulated net' and identifies it as glial cell cytoplasm. A similar appearance is described by Pipa, Cook and Richards[4] in Bodian's protargol preparations of these ganglia. In preparations made by the osmium and ethyl gallate technique, Wigglesworth[5] describes a "glial lacunar system", in which the glial cells lie in extensive cavities, the contents of which are not stained in the preparations. He finds that comparable regions are found in all the thoracic and abdominal ganglia of *Periplaneta*. It appears, then, that this region in the ganglia is very sensitive to the action of fixatives.

The only group of substances which can be identified histochemically in this area are acid mucopolysaccharides. The reactions for proteins (coupling reaction, mercury nitrite and Sakaguchi tests) and lipids (sudan black) give negative results, and so does the periodic acid–Schiff test. This latter result is in agreement with the finding of Leblond, Glegg and Eidinger[6] that acid mucopolysaccharides are negative

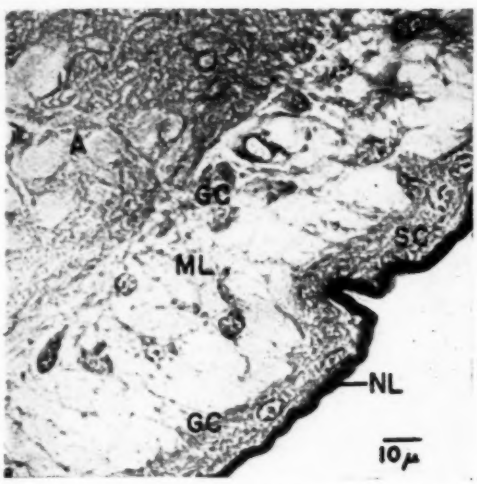

Fig. 1. Section through a thoracic ganglion of *Periplaneta americana* to show the region containing acid mucopolysaccharides. *A*, axons; *GC*, glial cells; *ML*, metachromatic layer; *NL*, neural lamella; *SC*, sheath cells

to the periodic acid–Schiff test. The metachromasy with toluidine blue is removed by incubation of the sections in hyaluronidase ('Hyalase'), but as this enzyme is not specific for hyaluronic acid, this does not give any information as to the nature of the acid mucopolysaccharide. An attempt is being made to characterize it biochemically.

The acid mucopolysaccharide forms a ground substance which resembles that of vertebrate connective tissue in its histochemical reactions, but the degree of chemical similarity remains to be determined. The function of this layer of acid mucopolysaccharide is not apparent. There are suggestions that acid mucopolysaccharides can act as ionic barriers allowing cations to pass through, but restricting the passage of anions[7]. But it seems unlikely that this acid mucopolysaccharide forms an ionic barrier, both because it is restricted to the ganglia and because removal of the connective tissue sheath from either the ganglia or connectives results in the blockage of nerve conduction[8]. Wigglesworth[5] suggests that the region might serve as a pool for nutrients, or as a means of maintaining the outer shape of the ganglion as changes in the state of nutrition alter the size of the cellular components of the ganglion. But, until more is known of the chemistry of this substance, it will be difficult to ascertain its function.

I am very grateful to Dr. A. G. Richards for his criticism of this communication.

DOREEN E. ASHHURST*

Department of Zoology,
The University, Manchester, 13.

* Present address: Department of Entomology and Economic Zoology, University of Minnesota, St. Paul, 1, Minnesota, U.S.A.

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Experimental Transmission of *Borrelia anserina* (Sakharoff 1891) by *Aedes aegypti*

THE causal organism of spirochætosis, *Borrelia anserina*, was first reported from geese in the Caucasus, and has since been isolated from the blood of infected geese, turkeys, ducks, fowls, partridges, crows and sparrows from all parts of Africa, Australia, Austria, Bulgaria, Brazil, Egypt, East Indies, Germany, Greece, Hungary, India, the U.S.S.R., Rumania and Turkey.

The known common vectors of spirochætosis are various species of *Argas* and *Ornithodoros*.

Recent outbreaks in the Kaduna area of Northern Nigeria gave indications that the disease could be transmitted by other blood-sucking insects, and it was therefore decided to attempt experimental transmission of spirochætosis with *Aedes aegypti* as this was readily available and was known to feed well on experimental birds. Laboratory-bred females were placed in plastic tubes with mosquito netting fixed at each end. This tube was held firmly on a bird (Rhode Island Red) that showed spirochætes in a stained thin blood film. After approximately 45 min. the tube was removed and the fully fed *A. aegypti* were separated from the unfed. After starving for several days they were then fed on clean birds. These birds were transferred to separate cages and thin blood films were taken daily. Control birds of the same age and breed were maintained under the same conditions and thin blood films were also taken daily from these birds.

The birds on which the *A. aegypti* had been fed developed a fever, became anæmic and showed spirochætes in their blood.

The control birds were normal throughout the experiment. Similar experiments are being carried out with *Culex fatigans*.

J. A. ROBERTS

Investigation and Training Centre,
Ministry of Animal Health and Forestry,
Kaduna,
Northern Nigeria.

PSYCHOLOGY

Cross-modal Transfer in Man

LITTLE is known about transfer between different sensory systems during learning of complex material. Positive cross-modal transfer implies that learning to discriminate between a pair of stimuli in one modality leads to accelerated discrimination between the same stimuli presented in a second modality. Previous experiments showed no evidence for transfer between visual and tactile[1], or visual and auditory[2], discrimination habits in monkeys, although one investigation has been reported recently in which transfer between auditory and visual habits was obtained[3]. In certain conditions, human subjects are able to match geometrically equivalent contours successively presented for visual and tactile comparison[4]. Auditory 'flutter' can similarly be matched with visual 'flicker' by subjects specifically instructed to equate rates of intermittance of sounds and lights[5]. Such cross-modal comparisons, however, are performed more poorly than intra-modal comparisons. A general theoretical argument against the immediate equivalence of perception in differing sense modalities has been presented by Hebb[6].

Table 1. Different Learning Sequences used with Four Groups of Subjects

Group I ($N=6$)	Group II ($N=6$)	Group III ($N=12$)	Group IV ($N=12$)
VISUAL	Auditory	Alternating	Matching
		VISUAL and auditory	auditory vs. visual
● – – vs. – – ● (+) (–)	····· vs. · · · (+) (–)	● – – vs. – – ●	● – – vs. – – ●
followed by	followed by		● – – vs. ● – –
auditory	VISUAL	● – – vs. – – ● (+) (–)	– – ● vs. – – ● – – ● vs. ● – –
● – – vs. – – ● (+) (–)	● – – vs. – – ● (+) (–)		

In the rhythm discriminations temporal parameters were as follows: Duration of short components, 148 msec.; duration of long components, 504 msec.; duration of intervals between components, 110 msec.; duration of intervals between rhythm cycles, 990 msec. In the rate discrimination, the sound was interrupted 10 times per sec. for the positive stimulus, and 4 times per sec. for the negative stimulus.

In the present work cross-modal transfer of rhythm discrimination by human subjects was investigated. We wished to determine, first, whether learning of a rhythm discrimination in one modality (audition) leads to more rapid learning of the same rhythm discrimination in the second modality (vision); secondly, we investigated the ability to match auditory and visual rhythms.

The subject sat facing a pair of boxes, each of which contained a filament lamp and loudspeaker. Luminance of the lamp was 230 ft. lamberts. The basic sound source was a 400-c.p.s. tone with a small component at 2,500 c.p.s. Its intensity was judged to be about 60 db. An electro-mechanical timing device interrupted the lights and sounds to produce the rates and rhythms described in Table 1. On each trial, stimuli were successively presented on the left and right; two cycles of the rhythms were always presented, whereas the intermittent stimuli were presented for 5 sec. The position of the positive stimulus, to left or right, was determined by reference to the Gellerman schedule. Testing continued, in all conditions, until the subject made no errors in 10 consecutive trials. By convention, the final 10 trials are not included in the scores reported here.

Thirty-six adult subjects were divided into four groups and tested according to the design shown in Table 1. A pilot experiment indicated that the rhythm discrimination was more difficult in the visual than in the auditory form. For this reason it was decided to study cross-modal transfer only from audition to vision. Group I ($N=6$) received no auditory training and group II ($N=6$) learned an irrelevant discrimination of the auditory rate before both groups learned the visual rhythm discrimination. Group III ($N=12$) learned the auditory and visual forms of the rhythm discrimination concurrently, the same pair of rhythms being given in auditory and visual form on alternate trials. None of the subjects in the first three groups was informed of the relationship between the auditory and visual rhythms. By contrast, the subjects in group IV ($N=12$) were specifically instructed to determine, on each trial, whether a visual rhythm was identical with an auditory rhythm which immediately preceded it.

The scores of subjects in groups I and II for the visual rhythm discrimination were combined ($N=12$) and then compared with the scores for the same discrimination of subjects in group III and also with the auditory-visual matching performance of subjects in group IV. Subjects in the first two groups required an average of 13·5 trials (range 0–57 trials) to learn the visual rhythm discrimination in the absence of associated auditory learning. Subjects in group III required an average of 20·4 trials to reach criterion on the visual rhythm discrimination (range 1–120 trials). Half the trials for these subjects, it must be noted, consisted of presentation of the auditory form of the rhythm discrimination. If these auditory trials are excluded, the average for group III becomes 10·3 trials (range 0–60 trials). Comparison of these test scores by means of the Mann-Whitney 'U' test failed to yield any significant differences between the groups, regardless of whether the auditory trials of group III were combined with the visual trials or not. Further analysis, using a less stringent performance criterion (90 per cent, instead of 100 per cent of 10 trials), again failed to reveal significant differences between the various groups. Finally, successful matching of the auditory and visual rhythms by subjects in group IV required an average of 8·4 trials (range 0–34 trials). This value does not differ significantly from the learning scores of the first three groups.

Another measure of the degree to which learning of one form of the discrimination influences learning of the other may be obtained by determining the difference in number of trials required by subjects in group III to learn the two forms of the discrimination. The average difference between auditory and visual learning scores of these subjects was 7·3 trials (range 1–56 trials). Three subjects showed such rapid learning of both auditory and visual forms of the discrimination (that is, fewer than five trials of each) that we would attribute these low difference scores to equally rapid concurrent learning in the two modalities. The visual learning scores given by 3 of the remaining 4 subjects with low difference scores, when calculated on the basis of combined auditory and visual trials, exceed the average visual learning score of control subjects. The other five subjects in group III showed large difference scores, suggesting little, if any, transfer between modalities.

With our conditions of testing, therefore, we have found no evidence for transfer from auditory to visual learning of rhythms in man. This result agrees with the finding of relatively poor cross-modal matching performance in human subjects[5]. If confirmed and extended, results such as ours might imply that discrimination learning of complex material is largely modality-specific.

The apparatus used in this experiment was designed and constructed by Mr. H. B. Morton.

M. Cole*
S. L. Chorover†
G. Ettlinger

Psychological Laboratory,
Institute of Neurology,
Queen Square,
London, W.C.1.

* Fellow of the National Multiple Sclerosis Society.
† Fellow of the National Foundation.

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FORTHCOMING EVENTS

Wednesday, September 20

INSTITUTE OF PETROLEUM (at 61 New Cavendish Street, London, W.1), at 5.30 p.m.—Dr. W. Domzalski: "Airborne Techniques in Petroleum Exploration".

Thursday, September 21—Friday, September 22

ROYAL ENTOMOLOGICAL SOCIETY OF LONDON (at the Imperial College of Science and Technology, South Kensington, London, S.W.7)—Symposium on "Insect Polymorphism".

APPOINTMENTS VACANT

APPLICATIONS are invited for the following appointments on or before the dates mentioned:

MYCOLOGIST (with an honours degree in botany, with specialization in plant pathology) at the States' Experimental Station (Department of Agriculture)—The Establishment Officer, States' Greffe, Jersey, C.I. (September 23).

RESEARCH DEMONSTRATOR (graduate in botany) to engage in research, preferably in plant physiology and biochemistry, plant pathology and ecology; and a RESEARCH ASSISTANT (graduate in botany or graduating this year), to assist the Professor in the establishment and maintenance of a collection of plant organ and tissue cultures and in research involving these cultures—The Registrar, University College, Singleton Park, Swansea (September 23).

LECTURER IN THE DEPARTMENT OF ELECTRICAL ENGINEERING—The Registrar, The University, Leeds (September 25).

PROFESSORS OF CROP HUSBANDRY AND HORTICULTURE; ANIMAL HUSBANDRY AND HEALTH; and AGRICULTURAL ECONOMY at the University of Ghana—The Assistant Registrar (London), University of Ghana, 15 Gordon Square, London, W.C.1 (September 25).

SCIENTIFIC OFFICER or SENIOR SCIENTIFIC OFFICER (physicist, chemical or mechanical engineer), to join a group studying the growth and spread of fire in and between buildings—The Director, Fire Research Station (D.S.I.R.), Borehamwood, Elstree, Herts (September 25).

LECTURER (with at least an honours degree in geology, together with special qualifications to teach geomorphology to the fourth year (honours) level in the Department of Geography) IN GEOGRAPHY AND GEOLOGY at the University of Natal, Pietermaritzburg—The Secretary, Association of Universities of the British Commonwealth (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (South Africa and London, September 30).

RESEARCH ASSISTANT (graduate or postdoctoral), to work on rapid high temperature reaction kinetics, fundamental flame properties, flame spectroscopy and allied subjects—Dr. G. Dixon-Lewis, Houldsworth School of Applied Science, The University, Leeds 2 (September 30).

RESEARCH ASSISTANT (with a degree in botany or equivalent qualifications) IN THE DEPARTMENT OF BOTANY—Dr. A. G. Smith, Department of Botany, The Queen's University of Belfast, Belfast 7 (September 30).

SCIENTIFIC OFFICER or SENIOR SCIENTIFIC OFFICER (with research experience in enzymology and/or protein chemistry, and some knowledge of, and interest in, fundamental genetic problems), initially to assist in current research on the effects of mutation on the structure of specific proteins in the mould *Neurospora crassa*—The Secretary, John Innes Institute, Bayfordbury, Hertford, Herts (September 30).

VICE-PRINCIPAL (with a good university degree and preferably higher qualifications and Associate Membership of one of the engineering institutions, and a sound technical background and experience in a similar capacity) of the Khartoum Technical Institute, Sudan—The Appointments Section, Sudan Embassy, 3 Cleveland Row, St. James's, London, S.W.1 (September 30).

VOKES RESEARCH FELLOW (preferably with a special interest in fluids or elastic theory) IN THE DEPARTMENT OF MECHANICAL ENGINEERING—The Secretary and Registrar, The University, Southampton (September 30).

LECTURER or ASSISTANT LECTURER (preferably with special qualifications and interests in cytology and genetics, or in protozoology, or in the ecology of terrestrial animals) IN THE DEPARTMENT OF ZOOLOGY, University of Ghana—The Assistant Registrar (London), University of Ghana, 15 Gordon Square, London, W.C.1 (October 2).

LECTURER IN BOTANY AND ASSISTANT CURATOR OF THE BOLUS HERBARIUM, University of Cape Town, South Africa—The Secretary, Association of Universities of the British Commonwealth (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (South Africa and London, October 4).

LECTURER IN PHILOSOPHY; and a LECTURER IN PSYCHOLOGY at the University of Kong Hong—The Secretary, Association of Universities of the British Commonwealth (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (Hong Kong and London, October 13).

CHAIR OF CHEMISTRY in the Royal College, Nairobi—The Secretary, Inter-University Council for Higher Education Overseas, 29 Woburn Square, London, W.C.1 (October 15).

LECTURER (with a science degree specializing in geology and research experience to Ph.D. or comparable level, and well trained in modern mineralogical techniques) IN GEOLOGY at the University of Queensland, Australia—The Secretary, Association of Universities of the British Commonwealth (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (Australia and London, October 15).

READER IN HUMAN GENETICS IN THE DEPARTMENT OF ZOOLOGY, University of Melbourne, Australia—The Secretary, Association of Universities of the British Commonwealth (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (October 15).

LECTURER (with a veterinary qualification) IN ANIMAL HUSBANDRY IN THE SCHOOL OF VETERINARY MEDICINE—The Registrar, Trinity College, Dublin, Republic of Ireland (October 16).

READER IN MICROBIOLOGY at the University of Adelaide, Australia—The Secretary, Association of Universities of the British Commonwealth (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (Australia, October 16).

SENIOR LECTURER IN PHYSICS at the Australian National University, Canberra—The Secretary, Association of Universities of the British Commonwealth (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (Australia and London, October 21).

SENIOR LECTURER (preferably with research experience in low temperature physics) IN PHYSICS at the University of Western Australia—The Secretary, Association of Universities of the British Commonwealth (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (Australia and London, October 21).

SCIENTIFIC OFFICER (with a first- or second-class honours degree in physics), to work on transmission of sound through panels and solid structures—The Director, Building Research Station (D.S.I.R.), Garston, near Watford, Herts, quoting Ref. BRS.32Y (October 24).

TEACHING FELLOWS (with an appropriate honours degree) IN THE DEPARTMENT OF BIOCHEMISTRY (two appointments); and in the DEPARTMENT OF BOTANY (one appointment), University of New South Wales, Australia—The Agent-General for New South Wales, 56 Strand, London, W.C.2, and the Appointments Section, University of New South Wales, Box 1, Post Office, Kensington, New South Wales, Australia (October 31).

LECTURER IN EDUCATION in the University of Otago, Dunedin, New Zealand—The Secretary, Association of Universities of the British Commonwealth (Branch Office), Marlborough House, Pall Mall, London, S.W.1, or The Registrar, University of Otago (London and New Zealand, November 20).

ASSISTANT LECTURER (graduate in chemistry or agricultural chemistry) IN SOIL SCIENCE—The Registrar, Massey Agricultural College (University of New Zealand), Palmerston North, New Zealand (November 22).

LECTURER or SENIOR LECTURER IN PHYSIOLOGY at Monash University, Melbourne, Australia—The Secretary, Association of Universities of the British Commonwealth (Branch Office), Marlborough House, Pall Mall, London, S.W.1, or The Registrar, Monash University (Australia and London, December 4).

AGRONOMIST (Soils) (male, between 26–40, with an honours degree in agricultural or horticultural science with agricultural chemistry as the main subject, experience in field experimental work on soil fertility, and a knowledge of tropical soils and crops) IN THE DEPARTMENT OF AGRICULTURE, Sarawak, to design and conduct field experiments with tropical crops on selected soils to assess the potentiality of the soils—The Director of Recruitment, Department of Technical Co-operation, Carlton House Terrace, London, S.W.1, quoting RC.213/128/01/T.

ASSISTANT LECTURER, Grade B (well qualified in physics and an interest in the development of some branch of applied physics) IN PHYSICS—The Clerk to the Governors, South-East Essex Technical College, Longbridge Road, Dagenham, Essex.

ASSISTANT (preferably with previous training), for work in cytogenetics—The Registrar (BX), Queen Mary College (University of London), Mile End Road, London, E.1.

PHYSICAL CHEMIST, Senior Scientific Officer grade (at least 26, with a degree or Dip. Tech. with first- or second-class honours degree in chemistry or equivalent qualification, and at least three years postgraduate research experience) at the Rocket Propulsion Establishment, Westcott, Bucks, to lead a small team investigating properties of materials at very high temperature with special emphasis on high temperature-resistant ceramics—The Ministry of Labour, Technical and Scientific Register (K), 28 King Street, London, S.W.1, quoting Ref. F.531/iA.

POSTDOCTORAL FELLOW IN ORGANIC CHEMISTRY—Dr. J. I. G. Cadogan, Department of Chemistry, King's College (University of London), Strand, London, W.C.2.

PROFESSOR OF ANALYTICAL CHEMISTRY at Peshawar University—The Department of Technical Co-operation, 26/28 King Street, London, S.W.1, quoting TCS/PAK/529.

READER IN APPLIED MATHEMATICS; a SENIOR LECTURER IN MATHEMATICAL STATISTICS; and a SENIOR LECTURER or LECTURER IN ANY BRANCH OF PURE OR APPLIED MATHEMATICS at the University of Adelaide, Australia—The Secretary, Association of Universities of the British Commonwealth (Branch Office), Marlborough House, Pall Mall, London, S.W.1, or The Registrar, University of Adelaide.

RESEARCH ASSISTANT (science or engineering graduate), to work on problems related to food processing or food engineering, and to undertake some teaching—The Principal, National College of Food Technology, St. George's Avenue, Weybridge, Surrey.

RESEARCH TECHNICIAN (with some experience in a biochemical research laboratory) IN THE DEPARTMENT OF CHEMISTRY AND FOOD TECHNOLOGY, for work on the isolation and chemical studies of lipoproteins from cereals and oilseeds—The Secretary, Borough Polytechnic, Borough Road, London, S.E.1.

SENIOR RESEARCH ASSISTANT and a RESEARCH ASSISTANT IN THE CHEMISTRY DEPARTMENT, to work on optical rotatory dispersion—Prof. W. Klyne, Westfield College (University of London), Hampstead, London, N.W.3.

TECHNICIANS and JUNIOR TECHNICIANS (young men or women with an interest in synthetic organic chemistry or in biochemistry)—The Secretary (LA), Imperial Cancer Research Fund, Lincoln's Inn Fields, London, W.C.2.

REPORTS and other PUBLICATIONS

(*not included in the monthly Books Supplement*)

Great Britain and Ireland

Royal Observatory Bulletins. No. 34: Design and Tests of a Mirror Transit Circle Axis. By R. d'E. Atkinson. Pp. A51–A70. (London: H.M. Stationery Office, 1961.) 4*s.* net. [127

Memoirs of the Royal Astronomical Society. Vol. 68, Part 3: Classification and Radial Velocities of Bright Southern Galaxies. By Gerard de Vaucouleurs and Antoinette de Vaucouleurs. Analysis

of Limb Darkening Observations. By A. Keith Pierce and John H. Waddell. Pp. 69–112. (London: Royal Astronomical Society, 1961.) 20*s*.; 3 dollars. [127

United Kingdom Atomic Energy Authority. Seventh Annual Report for the period 1st April, 1960—31st March, 1961. Pp. viii+80+4 plates. (London: H.M. Stationery Office, 1961.) 5*s*. net. [127

Scottish Plant Breeding Station. Report, 1961. Pp. 78+1 plate. Pentlandfield, Roslin: Scottish Plant Breeding Station, 1961.) [137

General Register Office. The Registrar General's Statistical Review of England and Wales for the two years 1957–1958. Supplement on Mental Health. Pp. ix+102. (London: H.M. Stationery Office, 1961.) 7*s*. 6*d*. net. [137

The British Coke Research Association. Seventeenth Annual Report for the year 1960. Pp. 33. (Chesterfield: The British Coke Research Association, 1961.) [147

Durham University Appointments Board. Fifteenth Annual Report. Pp. 9. (Durham: Durham University Appointments Board, 1961.) [177

Food for the Chemist. By Prof. S. H. Harper. (An Inaugural Lecture given in the University College of Rhodesia and Nyasaland on 28 October 1960.) Pp. 16. (London: Oxford University Press, 1961.) 4*s*. net. [177

Regional Advisory Council for Technological Education: London and Home Counties. Regional Development Plan for Courses for the Diploma in Technology. Pp. 16. (London: Regional Advisory Council for Technological Education: London and Home Counties, 1961.) [277

Department of Scientific and Industrial Research. Report of the Warren Spring Laboratory 1960: Report of the Warren Spring Laboratory Steering Committee and the Report of the Director of the Warren Spring Laboratory for 1960. Pp. vi+25+4 plates. (London: H.M. Stationery Office, 1961.) 3*s*. net. [277

B.B.C. Engineering Monograph No. 37: An Instrument for Measuring Television Signal-to-Noise Ratio. By S. M. Edwardson. Pp. 23. (London: H.M. Stationery Office, 1961.) 5*s*. [277

Horticultural Marketing Council. Fifth Annual Report and Statement of Accounts for the period 9th May, 1960 to 31st March, 1961. Pp. 16+4 plates. (London: Horticultural Marketing Council, 1961.) [277

Society of Chemical Industry. Proceedings of the Chemical Engineering Group, Vol. 39, 1958/9. Pp. viii+111. (London: Chemical Engineering Group, Society of Chemical Industry, 1961.) 42*s*. [277

Philosophical Transactions of the Royal Society of London. Series B: Biological Sciences. No. 705, Vol. 243 (20 July 1961): Different Types of Cirral Activity of Barnacles. By D. J. Crisp and A. J. Southward. Pp. 271–308+plate 66. 15*s*. No. 706, Vol. 244 (27 July 1961): Studies on Triassic Fossil Plants from Argentina. 1: *Rhexoxylon* from the Ischigualasto Formation. By S. Archangelsky and D. W. Brett. Pp. 1–19+plates 1 and 2. 9*s*. 6*d*. No. 707, Vol. 244 (27 July 1961): The Layered Ultrabasic Rocks of South-West Rhum, Inner Hebrides. By W. J. Wadsworth. Pp. 21–64+plates 3–7. 22*s*. 6*d*. (London: Royal Society, 1961.) [277

Problems of Psychology, Numbers 1 and 2 (1960). Published quarterly, containing selected papers translated from *Voprosy Psikhologii*. Four issues of approximately 400 pages per annum. Annual subscription: (A) For libraries £15 (40 dollars); (B) For private subscribers £5 (15 dollars). Single issues £4 (12 dollars). (London and New York: Pergamon Press, 1961.) [277

Glamorgan Forests. Edited by H. L. Edlin. (Forestry Commission Guide.) Pp. vi+99+20 plates. (London: H.M. Stationery Office, 1961.) 5*s*. net. [277

Department of Scientific and Industrial Research. Proceedings of Conference on Ergonomics in Industry, 27–29 September 1960. Pp. x+190. (London: H.M. Stationery Office, 1961.) 12*s*. 6*d*. net. [277

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General Register Office. The Registrar General's quarterly return for England and Wales—Births, Deaths and Marriages; Infectious Diseases; Weather; Population Estimates, No. 449, 1st Quarter 1961. Pp. 28. (London: H.M. Stationery Office, 1961.) 2*s*. 6*d*. net. [277

Freshwater Biological Association. Twenty-ninth Annual Report for the year ended 31 March 1961. Pp. 88. (Ambleside: Freshwater Biological Association, 1961.) 4*s*. [277

The British Ceramic Research Association. Thirteenth Annual Report, 1960. Pp. 70. Special Publication No. 27: Symposium on Dust Control in the Pottery Industry, 8th April, 1960. Pp. 95. (Penkhull, Stoke-on-Trent, 1961.) [277

United Kingdom Atomic Energy Authority. Atom 1961: an Illustrated Summary of the Seventh Annual Report from 1st April 1960 to 31st March 1961. Pp. 36. (London: H.M. Stationery Office, 1961.) 2*s*. 6*d*. net. [277

United Kingdom Atomic Energy Authority. Dounreay Experimental Reactor Establishment, Thurso, Caithness—Information Booklet. Pp. 59. (London: United Kingdom Atomic Energy Authority.) [277

Government of Northern Ireland: Ministry of Commerce. Memoirs of the Geological Survey. Geology of the Country around Dungannon. (One-inch Geological Sheet 35.) By A. Fowler and J. A. Robbie. With an Appendix on Geophysical Investigations by Dr. W. Bullerwell. Second edition of Explanatory Memoir by E. T. Hardman. Pp. xii+274+14 plates. (Belfast and London: H.M. Stationery Office, 1961.) 30*s*. net. [277

Other Countries

Iceland. The University Research Institute—Department of Fisheries. Rit Fiskideildar. Vol. 2, Nr. 9: The Salinity at the Shores of Southwest Iceland. By U. Stefansson. B. Lindal, J. Jakobsson, and I. Jonsson. Pp. 26. Vol. 2, Nr. 10: Icelandic Driftnet Herring Tagging Experiments. By J. Jakobsson. Pp. 16. (Reykjavik: University Research Institute—Department of Fisheries, 1961.) [277

Académie Royale de Belgique. Classe des Sciences. Mémoires. Tome 32, Fascicule 6: Les Deux Flores d'Afrique Tropicale. Par J. Lebrun. Pp. 81. (Bruxelles: Académie Royale de Belgique, 1961.) 70 francs. [277

Canada: Department of Northern Affairs and National Resources. Bulletin No. 172 (Biological Series, No. 65): Contributions to Zoology, 1959. Pp. iv+135. 1 dollar. Natural History Papers. No. 9 (April 13, 1961): Bird and Mammal Observations on Ellef Ringnes Island in 1960. By D. B. O. Savile. Pp. 6. No. 10 (April 13, 1961): Noteworthy Records of Marine Molluscs from the Bay of Fundy. By E. L. Bousfield. Pp. 3. (Ottawa: Queen's Printer, 1961.) [277

Thirteenth Annual Report of the Pacific Marine Fisheries Commission for the year 1960. Pp. 60. (Portland, Oregon: Pacific Marine Fisheries Commission, 1961.) [277

East Africa High Commission. East African Virus Research Institute Report, July 1959–June 1960. Pp. i+57. (Entebbe: East African Virus Research Institute, 1960.) [277

Hungary. Year-Book of the Institute of Nutrition, Budapest, for 1960. Pp. iv+88. (Budapest: Institute of Nutrition, 1961.) [277

Canada: Department of Mines and Technical Surveys. Geological Survey of Canada. Map 42–1960: Drift-Thickness Contours—Montreal Area, Quebec. Map 1089A: Perth, Lanark and Leeds Counties, Ontario. (Ottawa: Geological Survey of Canada, 1960 and 1961.) [277

Institut pour l'Encouragement de la Recherche Scientifique dans l'Industrie et l'Agriculture. Rapport Annuel Exercise 1960. Pp. 255. (Bruxelles: Institut pour l'Encouragement de la Recherche Scientifique dans l'Industrie et l'Agriculture, 1961.) [277

International Atomic Energy Agency, Vienna. IAEA—What It Is and What It Does. Pp. 16. (Vienna: International Atomic Energy Agency, 1961.) [277

Commonwealth of Australia: Department of External Affairs. Australian National Antarctic Research Expeditions. ANARE Reports, Series B, Vol. 3: Oceanography, Hydrology. Introductory Studies: Hydrology and Plankton, Mawson, June 1956—February 1957. By J. S. Bunt. Pp. 135 (9 plates). (Melbourne: Antarctic Division, Department of External Affairs, 1960.) [277

Commonwealth of Australia. Research Review of the Commonwealth Scientific and Industrial Research Organization for the year ended 30th June, 1960. Pp. iv+418. (Melbourne: Commonwealth Scientific and Industrial Research Organization, 1961.) [277

Geological Survey of British Guiana. Quarter Degree Sheet 0660 S.E.: Apaiqua S.E. Edited by E. Williams. (Georgetown: Geological Survey, 1961.) 1 dollar. [277

Académie Royale de Belgique. Classe des Sciences. Mémoires. Tome 32, Fascicule 7: Hydrogénations Électrochimiques et Catalyse. Par Xavier de Hemptinne. Pp. 44. (Bruxelles: Académie Royale de Belgique, 1961.) 70 francs. [277

New Zealand Forest Service: Forest Research Institute. Technical Paper No. 33: Measurement of Stand Basal Area with Optical Wedges. By W. G. Warren. Pp. 12. (Wellington: Goverment Printer, 1960.) [277

Federation of Malaya. Report on Forest Administration for the year 1959. By Mohd. Alwy bin Haji Suleiman. Pp. iv+117+2 plates. (Kuala Lumpur: Goverment Printer, 1961.) 2.50 Malayan dollars. [277

United States Department of Agriculture. Publication Pa–436: The Khapra Beetle—a Pest of Stored Grains and Cereal Products. Pp. 8. (Washington, D.C.: Government Printing Office, 1961.) [277

Report and Accounts of the National Botanic Gardens of South Africa, for the financial year ended 31 March, 1961. (Kirstenbosch, Newlands, Cape; Karoo Garden, Worcester; and Harold Porter Botanic Reserve, Betty's Bay.) Pp. 27. (Kirstenbosch, Newlands, Cape: National Botanic Gardens of South Africa, 1961.) [277

Records of the Australian Museum. Vol. 24, No. 13 (9th March, 1959): Cave Art of the Conjola District, New South Wales. By Frederick D. McCarthy. Pp. 191–202+plate 22. Vol. 24, No. 14 (9th March, 1959): Rock Engravings of the Sydney-Hawkesbury District. Part 2: Some Important Tirual Groups of the County of Cumberland. By Frederick D. McCarthy. Pp. 203–216+plates 23–26. Vol. 25, No. 1 (9th March, 1959): Some Australian Polyclads (Turbellaria). By Libbie H. Hyman. Pp. 1–17. Vol. 25, No. 2 (1st September, 1959): Serpulidae (Polychaeta) from Australia. By Barbara Dew. Pp. 19–56. Vol. 25, No. 3 (26th November, 1959): The Peloriidiidae of Lord Howe Island (Homoptera, Coleorrhyncha). By J. W. Evans. Pp. 57–62. (Sydney: The Australian Museum, 1959.) [277

Durban Museum Novitates. Vol. 6, Part 8 (30th June, 1961): Miscellaneous Taxonomic Notes on African Birds, XVII. By P. A. Clancey. Pp. 105–118. 30 cents net. Vol. 6, Part 9 (30th June, 1961): Revisionary Notes on the Southern and Eastern Races of the Yellow-Breasted Apalis *Apalis flavida* (Strickland) of Africa. By Walter J. Lawson. Pp. 118–126. 20 cents net. (Durban: Durban Museum, 1961.) [277

Annals of the South African Museum. Vol. 46, Part 5: A New Cichlid Fish of the Limpopo Basin. By E. Trewavas. Pp. 53–56. (Cape Town: South African Museum, 1961.) 10 cents. [277

East African High Commission. Annual Report of the East African Freshwater Fisheries Research Organization for 1960. Pp. iv+48. (Jinja, Uganda: East African Fisheries Research Organization, 1960.) Shs. 4. [277

Report of the South African Museum for the year ended 31st March, 1960. Pp. ii+19+4 plates. (Cape Town: South African Museum, 1961.) [277

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